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THE UNIVERSITY OF ALBERTA

THE PRODUCTION OF HIGH FRUCTOSE SYRUP FROM BEET MOLASSES

by

PO-WAH WONG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE

in

FOOD PROCESSING

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Supervisor

Date AUGUST 13th, 1982

DEDICATION

To my Mom and Dad

ABSTRACT

Diluted beet molasses (≈ 35% t.s. w/v) was decolourised by ultrafiltration and activated carbon, which removed 65.7 and 27.5% of the original colour, respectively. Approximately 55% of the original sugar content was lost during ultrafiltration and 6.0% during the activated carbon treatment. The resulting light-brown solution was further purified with ion exchange resins where 40.8% of the original ash content and 4% colour were removed. Complete hydrolysis of sucrose in the purified solution was obtained with a strong cation exchanger in the H^+ form. Sugar loss of 10.5% occurred during the hydrolysis. Partial separation of fructose from glucose in the hydrolysate was performed with a calcium-charged ion exchange resin, giving a resolution of 0.57. The glucose fraction was isomerised using a strong base (OH^- form) anion exchange resin. The isomerisation ratio obtained was 63.7%. The final product, a mixture of fructose fraction from the separation process and the isomerised product, contained 66% fructose by weight of total sugar. It had a clear light yellow colour, sweet taste with slight sour after-taste and a slight residual beet molasses odour. The total solids content of the product was 3.25%, thus concentration was required to convert it to high fructose syrup.

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1. - INTRODUCTION

Molasses is the end product of the sugar extraction process where no additional sugar can be economically recovered by direct crystallisation (McGinnis, 1951). The word 'molasses' is used to describe the residual syrup or 'mother liquor' left after the extraction of sucrose from the juices of sugar cane, sugar beets and other sucrose-containing materials (Keller, 1967). The molasses used in this research was 'final beet molasses', a viscous liquid obtained after three successive sugar crystallisations (Figure 1).

California beet molasses contains sucrose and raffinose, but practically no invert sugar (glucose and fructose). However, Alberta beet molasses contains significant amount of fructose. The typical compositions of final beet and cane molasses are shown in Table 1.

According to Keller (1967), the degree of exhaustion of sucrose in sugar manufacturing process is related to reducing sugar content in mother liquor. Higher amounts of reducing sugar in overall sugar concentration will exhaust sucrose from the mother liquor more completely. This explains the lower sucrose content in cane molasses, with higher concentration of invert sugars, as compared to beet molasses.

The greatest loss of sugar in a sugar factory operation is that which goes to the residual end product of molasses. Between 12 to 18% of the total sugar of beets is 'lost' to the molasses.

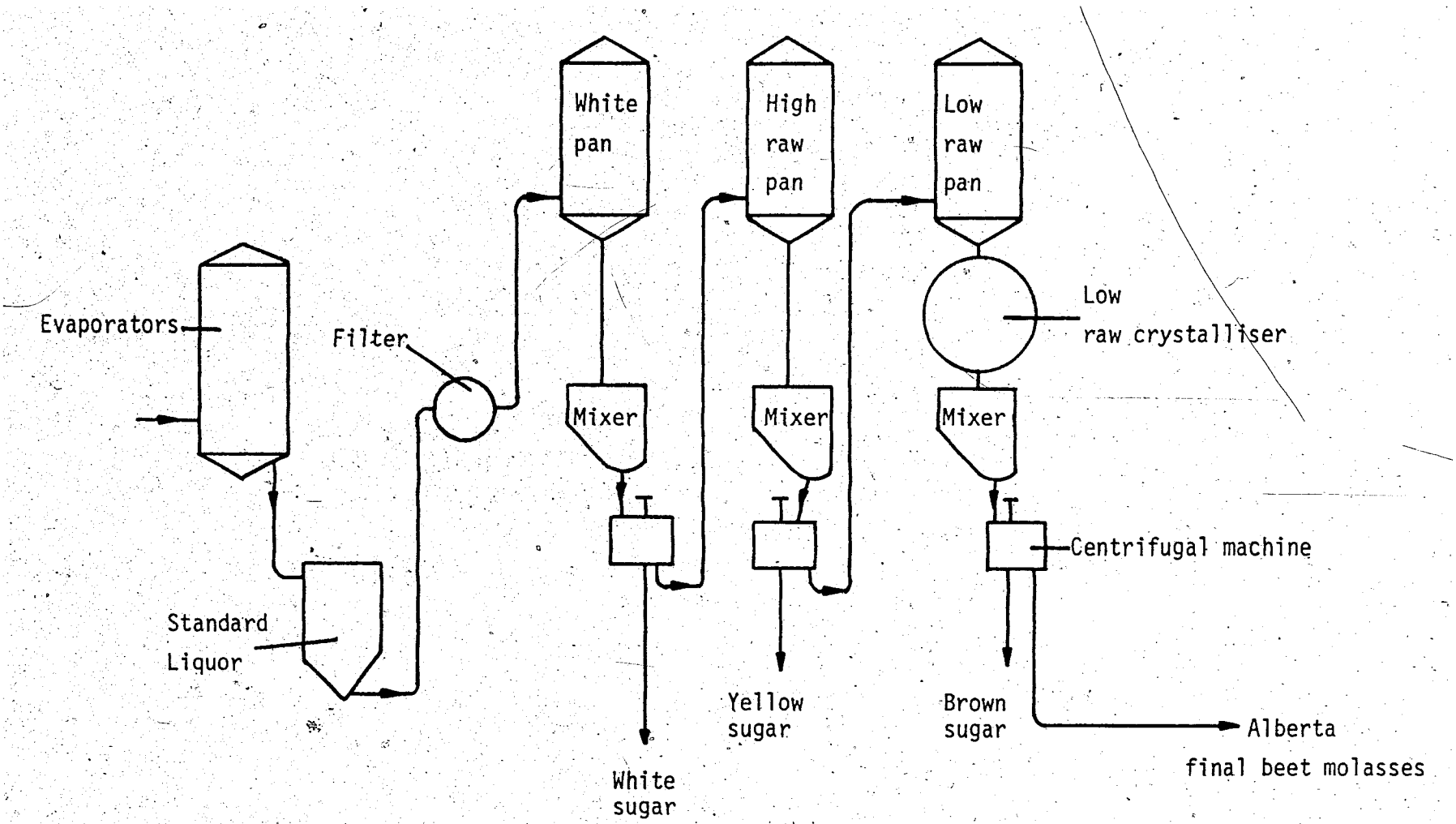


Figure 1. Diagram of the 'intermediate' stages of sugar manufacturing.

(Adapted from Canadian Sugar Factories, Lethbridge, Alberta.)

Table 1. Composition of beet and cane molasses

Type and source of molasses	Sucrose %	Raffinose %	Invert %	Moisture %	Ash %
Beet molasses[1]	51.0	1.0	1.0	16.50	11.50
Alberta beet[2] molasses	48.70 ± 1.35	—	9.61 ± 0.44 (fructose)	19.05 ± 0.49	3.06 ± 0.12*
Cane molasses[3]	30.0	—	10.0	17.0	7.0

1 McGinnis (1951).

2 1981 campaign.

3 Meade and Chen (1977).

* Conductivity measurements expressed as NaCl concentration.

Many processes and patents exist on the commercial recovery of sucrose from beet molasses. Some of the processes include: the Steffan Process, whereby sucrose is preciptated by calcium oxide as tricalcium saccharate in an insoluble granular form (McGinnis, 1951); the Barium Saccharate Process, whereby sucrose is precipitated as barium saccharate (McGinnis, 1951); and the Finnsugar-Pfeifer and Langen Molasses Desugarisation Process, whereby diluted molasses is separated into sugar and non-sugar fractions by ion exclusion with an organic cationic resin (Hongisto, 1977).

Beet molasses is used as a cattle feed supplement, usually mixed with dried pulp or beet tops, and for the production of alcohol, citric acid, yeast and monosodium glutamate (a flavour accentuator). Beet molasses is also an important source of many biochemicals (McGinnis, 1951).

For the 1981 - 82 Alberta beet crop, the projected production of final beet molasses is about 30,000 tons (Globe and Mail, October 27th., 1981). In Alberta final beet molasses is used for livestock feeding only. There is no further processing of this molasses.

In the production of animal feed, the final beet molasses is diluted to about 40° Brix and sprayed onto the partially dried pulp. The pulp is then dried and pelletised. Feed production does not utilise a significant portion of the molasses produced. Japan who is the major importer of Alberta beet by-products only imports the dried beet pulp. Much of the molasses produced is

stored in bulk storage tanks. There appears, therefore, to be definite need to develop alternate, commercially viable processes to utilise this sucrose-rich surplus molasses. This study was initiated to study a process in which the molasses may be converted to high fructose syrup which is a valuable form of sweetener for human foods.

In 1976, the world-wide consumption of sucrose came to nearly 81 million metric tons. Sweeteners are receiving increasing attention for many reasons, including world shortage, high prices, nutritional concerns, and consumer activism against high usage of refined sugar. Fluctuating sugar prices, the increasing production costs of sugar production and the increasing demand for sugar as standards of living in poorer countries rise, coupled with shortage of prime agricultural land for sugar-cane and sugar-beet cultivation, have forced the sweetener industry to look for alternative sweeteners.

Sweetener made from starch (dextrose and dextrose syrups) have been on the market for many years, but, due to their relatively low degree of sweetness (a maximum of 0.7 times the sweetness of sucrose), their use was restricted to certain applications where sweetening power was not the prime consideration.

High fructose syrup (HFS) is a commercial reality and competes favourably with sucrose in major food applications (Gramera, 1978). Increased fructose content results in

increased sweetness of the resulting syrup and also a reduction in the crystallisation problem since fructose does not crystallise as readily as dextrose. It is predicted that by the 1980's the United States will produce 6.5 billion pounds of HFS, consisting of product varieties with a fructose content ranging from 42 to 90% (Gramera, 1978).

HFS, also known as iso-syrup, can be used in practically every food in which a liquid sucrose or invert syrup is now employed. It was first introduced into the U.S. in 1967 on a small scale. With its acceptance by the food markets, its consumption rose rapidly.

The beverage industry is currently the largest user of HFS. However, other sectors of the food industry (eg. bakery and dairy) are making increased use of this product. One of the major reasons for this is that the product is sweeter and production costs are competitive with sucrose. Examples of the uses of HFS include preserves and marmalades, powdered beverage mixes, sweets and confections, frozen products, bakery products, ice cream, table-top sweeteners and cake mixes. Hence, the potential uses for fructose, whether in liquid or powder, are virtually limitless. The advantages of HFS are: higher sweetness (useful for diet formulations), high fermentability, low viscosity, clean non-masking taste, reduced tendency to crystallise compared to dextrose or sucrose (Mermelstein, 1975). Fructose is more readily tolerated by diabetics than is sucrose

(Schwimmer, 1981). Some of the disadvantages are high hygroscopicity and in high protein foods, an, excessively dark colour may develop from the reaction between reducing sugars and nitrogen compounds when the food is heated to high temperature (Mermelstein, 1975). Honey and fresh fruit juices are easily subjected to HFS adulteration. Also, fructose allegedly may contribute to heart disease (Schwimmer, 1981).

High fructose syrups has generally been marketed with the following composition: fructose 42%, glucose 50 - 55%, other sugars (lower oligosaccharides) 3 - 8% and dry matter 70 - 71%. At present there are three types of HFS on the market, namely 42, 55, and 90% syrups. The percentage indicates the amount of fructose in the total solids of these products. Enrichment processes, involving separation and recombination, are employed in the production of syrups with fructose content greater than 42%.

There are many sources of raw materials or starting materials for making HFS, e.g. corn, rice, potato, wheat, cassava (manioc), and even sucrose. The most common raw material currently used for the manufacture of HFS is corn starch from the corn wet milling industry. The term 'corn syrup' simply means that the syrup was made from corn starch as the original starting material. Hence, HFCS is an HFS made from corn starch. HFCS increased its share in the U.S. sweetener market from 10% to 22% between 1960 and 1978 (Gramera,

1978). The U.S. production of HFCS was predicted to reach as much as 10 billion pounds by 1980, replacing 30 to 40% of industrial sugars.

Regardless of the type of starch used as the starting material, the conversion process is basically the same and consists of the following three main steps: liquefaction, saccharification, and isomerisation. Each step of the above process is catalysed by a specific enzyme. For liquefaction (also known as dextrination), a bacterial alpha amylase is used. This enzyme randomly hydrolyses the alpha 1 - 4 linkages of starch to dextrins, intermediate starch units of 4 to 10 glucose residues. A bacterial amyloglucosidase (also known as glucoamylase) is employed in the saccharification process. This enzyme acts on the above dextrins splitting them into glucose molecules by acting on the alpha 1 - 4 and 1 - 6 linkages. Isomerisation employs another bacterial enzyme called glucose isomerase to catalyse the isomerisation or rearrangement of the glucose molecules to fructose. Figure 2 shows a flow diagram of high fructose syrup production from corn starch (American Maize Product Co., IN).

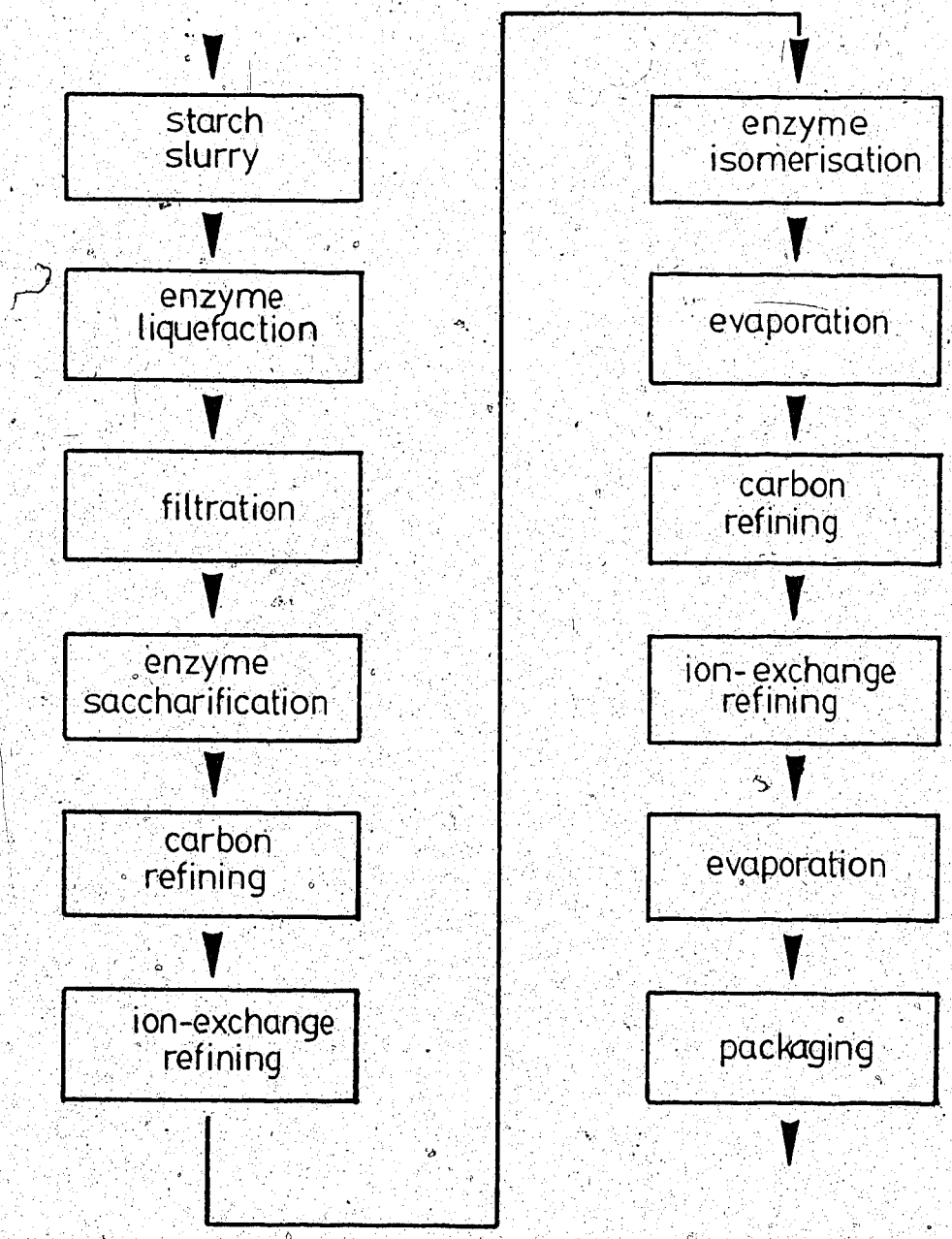


Figure 2. Flow chart of the process for the manufacture of HFS from corn starch.

(From American Maize Product Co., IN.)

2. OBJECTIVE OF THE INVESTIGATION

The objective of this research was to study the process through which beet molasses could be purified and converted to a product containing high concentration of fructose suitable for use as a food sweetener. The proposed process consisted of several unit operations as shown in the following diagram:

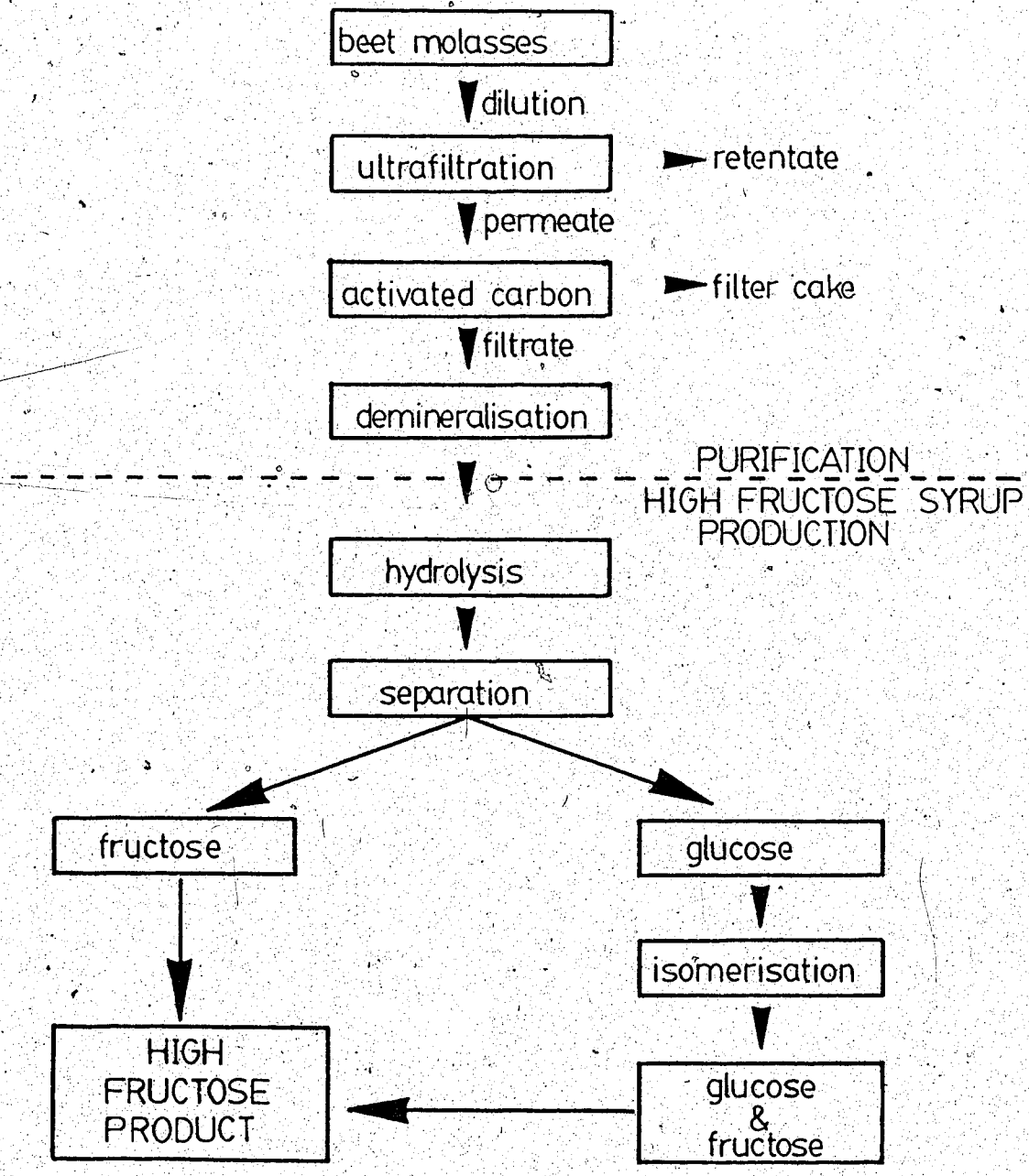


Figure 3. Flow chart of the proposed process for the production of HFS from Alberta beet molasses.

3. LITERATURE REVIEW

3.1 Impurities in Beet Molasses

Impurities in beet molasses can originate from three sources.

1. Constituents in the raw material which have gone through sugar manufacturing process unchanged and which cannot be removed economically, e.g., the so-called harmful nonsugar components or colloidal finely suspended nonsugars.

2. Constituents that originate or undergo changes during the manufacturing operations, e.g., the insoluble degradation products of sugars and proteins.

3. Nonsugars which become insoluble during the concentration and crystallisation processes as a result of their low solubility in highly concentrated solutions.

These constituents may be broadly grouped into proteinaceous and colloidal materials, ashes and colourants.

3.1.1. Proteinaceous and colloidal materials

The proteinaceous materials present in beet molasses are the sugar beet proteins which are extracted into the diffusion juice during the sugar extraction process. Albumins and other proteinlike substances are largely removed or precipitated in the defecation steps of the sugar manufacturing

process and only traces are present in the final molasses. The principal compounds are the molasses 'browning' products of amino acids, or proteins, and reducing sugars (Honig, 1963).

Molasses colloids, usually, are adsorption compounds formed from pectin-like materials and sugar. Due to the pectin content, most colloidal particles are found in the raw juice. Defecation removes colloidal particles in the early stages of the process, but their concentration again increases during the subsequent boiling steps, resulting in the accumulation of these particles in the molasses. Molasses colloids are 96% organic. The negatively charged colloids include the caramel substances and the melanoidins. They are the predominant colouring matters in molasses. The colloidal particles constitute about 0.3 to 0.5% in beet molasses and arises mainly from the liming and carbonatation steps of the sugar manufacturing process (Honig, 1963).

3.1.2. Inorganic components

Molasses contains a large variety of salts. These salts form the bulk of the inorganic nonsugars, the amounts and composition of which are influenced by beet varieties and the soil and weather conditions under which they are grown. Beet molasses contains about 10% ash, approximately 80% of which is made up of potassium and calcium and the remaining 20% consists of chlorides, sulphates, phosphates and silicates.

The minerals from raw beet juice are essentially passed into the final beet molasses. The use of lime in the defecation steps of sugar manufacturing process causes an increase in calcium content of the molasses. The following is a typical analysis of the ash of final beet molasses (Keller, 1967).

Calcium (%) as CaO	0.15 - 0.70
Potassium (%) as K_2O	2.20 - 4.50
Phosphate (%) as P_2O_5	0.02 - 0.07
Silica (%) as SiO_2	0.10 - 0.50
Magnesium (%) as MgO	0.01 - 0.10
Iron (%) as Fe_2O_3	0.001 - 0.02
Total Ash (%)	4 - 8

3.1.3. Colourants

Most of the colour formation in the sugar manufacturing process occurs towards the end of the process in the vacuum pans and crystallisers. High temperature (> 75° C) together with low purity and high density give rise to the secondary phases of the colour formation reactions, e.g., polymerisation. The majority of the colourants formed during the early part of the process is removed during the defecation step where milk of lime is added, followed by gaseous carbon dioxide to remove the added lime and colloidal impurities. Colours not removed by defecation, and those formed later in the process, end up

in the final molasses after repeated sugar crystallisations. Colour compounds present in beet or cane molasses can be classified as follows:

a. Melanoidins

These are polymerised products of amino acids, or amine-containing compounds and reducing sugars. The reaction is known as Maillard reaction. The products are dark brown polymeric substances (Binkley and Wolfrom, 1953) which may account for a large part of the observed colour even though they are present in small amounts.

b. Caramel

Caramel is produced mainly by the heating of sugar during the sugar manufacturing process. The degree of discolourisation is related to pH and temperature. The sequence of the colour formation can be generally summarised as follows:

Sucrose $C_{12}H_{22}O_{11}$
$\xrightarrow{-H_2O}$
Isosacchrosan (an anhydrosugar) $C_{12}H_{20}O_{10}$
$\xrightarrow{-H_2O}$
Caramelan $C_{24}H_{36}O_{18}$
$\xrightarrow{-H_2O}$
Caramelen $C_{36}H_{50}O_{25}$
$\xrightarrow{-H_2O}$
Caramelin (humins) $C_{125}H_{188}O_{80}$

Hence, caramel is actually a complex mixture of caramelan, caramelen, and caramelin (Honig, 1963).

c. Phenolics

Phenolics are the products of polymerised phenolic compounds present in beet juice. The polymerisation is catalysed by an enzyme, beet tyrosinase. Phenolics are usually found in combination with other compounds and seldom exist in beet molasses (McGinnis, 1951). An example of a polyphenolic coloured substance is beet tannin.

d. Iron-polyphenolic condensation compounds.

The preceeding polyphenolic compound can combine with iron to form intensely coloured iron complex. Some of these compounds survive the defecation process of liming and carbonatation, and darken further with prolonged exposure to air and ferric iron at elevated temperatures. Pyrocatechol which occurs in the epidermis and the head of beets, forms a complex with iron to give a yellow-greenish discolouration to the beet juice (Honig, 1953).

e. Beet pigments

In addition to coloured substances formed during the sugar manufacturing process, naturally occurring pigments such as betaine are also present in beet molasses.

This generalised description of the colourants present in beet molasses shows that the most important groups of colouring materials are caramel substances, melanoidins, and iron-polyphenolic compounds. These colourants are sensitive to

pH as their intensity increases with increasing pH (Meade and Chen, 1977).

One common property of sugar colourants is their ionic nature, making them conducive to ion exchange. Both basic and neutral coloured compounds were found to be present in the substances isolated from molasses (Tu and Payne, 1965). However, some of the colourants are zwitterionic, presenting difficulty when ion exchange technique is used for decolourisation. The empirical formula of the repeating units in the brown colourant isolated from molasses was found to be $C_{17-18}H_{26-27}O_{10}N$, containing amino acid and sugar moieties (Hsieh *et al.*, 1968). Molecular weight of beet molasses colourants could range from a few hundred to a hundred thousand.

It must be emphasised, therefore, that molasses colour is not due to any one substance; rather, it is the summation of an optical effect of a very large number of components, all present in trace amounts. For the most part, beet colourants have not been individually isolated and identified. Knowledge of their chemical composition has been derived largely from a comparison of similarities with prepared model systems (Tu and Payne, 1965). The main problem with the study of colourants is the difficulty in developing standard colourant solutions. Wavelength scan to identify the presence of characteristic groups have helped in giving clues as to the origin of these compounds (Agarwal and Misra, 1972a,b). Isolation of colourants by organic resins, dialysis and gel

electrophoresis have been helpful. However, only general conclusions could be drawn and that is that the major molasses colourants are composed of thermal degradation products of sugars, and sugar and amino compound reaction products (Yamane and Suzuki, 1963). Thus, decolourisation of molasses is by no means a simple process.

3.2 Purification of beet molasses

The preceeding section has shown that a considerable amount of impurities are present in beet molasses, especially colourants. It is necessary to remove these impurities before satisfactory HFS could be produced. Ultrafiltration, powdered activated carbon treatment and demineralisation are among the most efficient techniques which may be used for the purpose. Ultrafiltration assisted in the clarification of proteinaceous and colloidal material and the removal of colourants of high molecular weight. Powdered activated carbon removes carbon-adsorbable colourants. Demineralisation with ion exchange resins would reduce the ash content and some of the remaining colourants after activated carbon treatment. Together, the three operations would perform a satisfactory function of molasses purification.

3.2.1 Ultrafiltration

Ultrafiltration is a membrane separation process, a

physical process by which, under the influence of a driving force, a separation takes place across a semi-permeable membrane. An ultrafiltration set-up consists basically of two units: a module which houses the semi-permeable membranes and a high pressure pump which provides the driving force essential to effect the separation. The heart of an ultrafiltration unit is the membranes. Membranes used in ultrafiltration are uniquely structured films, typically in the 100 μm thickness region. Materials used to form these membranes are synthetic organic polymers such as cellulose acetate esters, polyamides and polysulfones. Special techniques such as phase-inversion solution casting are used for flat sheet membranes and spun casting are used for hollow fiber membranes (Cooper, 1980). For flat sheet membranes, the ultra-thin skin layer (about 0.2 μm), are superimposed on a coarsely porous matrix, usually plain filter paper, for mechanical strength and support. A measure of control over the degree of retention or selectivity of these membranes is possible by varying the casting conditions during their production. Good membrane characteristics are high water permeability, precisely controllable solute retention spectra and excellent thermal, chemical and mechanical durability. Operating variables in ultrafiltration, with a given set of membranes, are temperature, feed rate and pressure. Permeate flux decreases as the concentration of membrane-retained solids increases. This is due to the

increasing viscosity of the fluid and the concomitant increase in boundary layer thickness which increases resistance to permeate flow. Therefore, in each application, for technical and economic reasons, limits are imposed on the maximum attainable concentrations of the retained solids.

In ultrafiltration, the skin-layer of the membrane contains ultra-fine pores, usually in the 2 - 10 nm size range. These relatively fine pores retain macromolecules and colloids, while allowing the permeation of water and small molecules, e.g. sugars and inorganic salts. Retention of suspended particles is based on a sieving-type mechanism and depends on molecular size and shape of the particles. Ultrafiltration membranes are characterised with respect to their retention capabilities by specifying a nominal molecular weight cut-off. This is the minimum molecular weight which the membrane will retain.

The general equation describing flux ($L/m^2/hr$) in membrane processes (both ultrafiltration and reverse osmosis) is that cited by Jelen (1979):

$$J = K_p (\Delta P - \Delta \Pi) / (R_m + R_b)$$

where J = the flux through the membrane (permeation rate - $L/m^2/hr$)

ΔP = the hydrostatic pressure difference across the membrane.

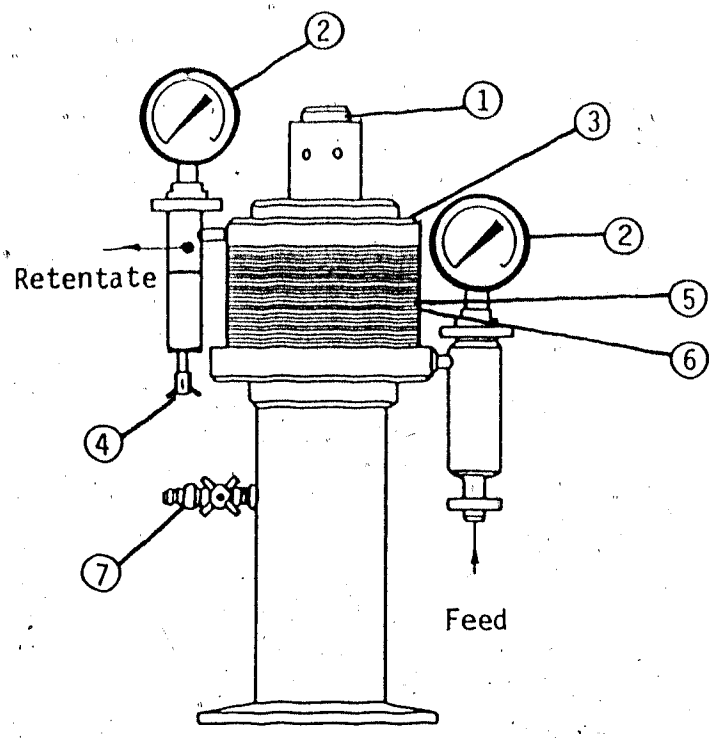
$\Delta \Pi$ = the osmotic pressure difference across the membrane.

R_m and R_b = the resistances to solvent permeation of the membrane and of the boundary layer of solutes attached to the membrane. (R_b may include the concentration polarisation and fouling effects.)

K_p = a proportionality constant related to mass transfer.

3.2.2 General designs, operations, and application to ultrafiltration.

Most ultrafiltration processes normally operate within the flux range of 1 - 100 $L/m^2/hr$ at pressures between 100 and 600 kPa, and temperature of about 60° C. The maximum flux is determined by the intrinsic membrane water flux which lies in the range of 100 - 500 $L/m^2/hr$. Several makes and models of ultrafiltration equipment are available on the market. They differ mainly in the shape and size of the membrane module but the principle is basically the same. The more common designs are the plate and frame stacks, hollow fiber tubes, spirally wrapped membranes, and flat-leaf cartridges, to name a few. The module used in this research was a plate and frame stack design from the Danish Sugar Corporation (DDS - De Danske Sukkerfabrikker, Naskov, Denmark) (Figure 4). Ultrafiltration systems can be operated either on a batch or continuous basis depending on processing conditions and availability of equipment. The systems can be further differentiated into either series or parallel operation (Figures 5 and 6). The



1. Center bolt
2. Pressure gauge
3. Top flange
4. Pressure regulating valve
5. Membrane support plate
6. Spacer plate
7. Hydraulic coupling

Figure 4. Diagram of the De Danske Sukkerfabrikker plate and frame LAB-MODULE 20 Model.
(Adapted from operation manual No. 1694 - GB - 0678 - 50.)

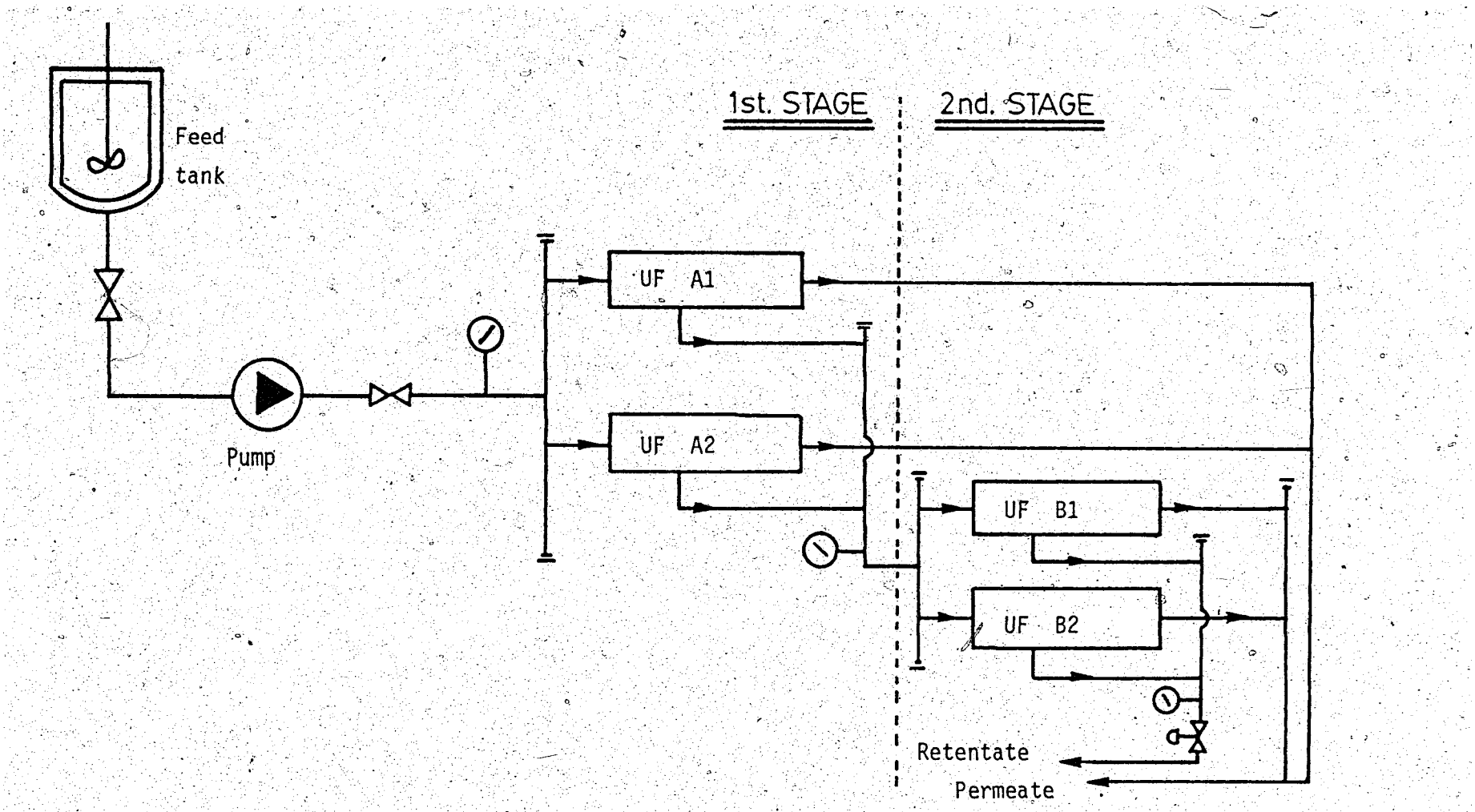


Figure 5. Diagram of an ultrafiltration process in a multi-stage series configuration. (Adapted from Cooper, 1981.)

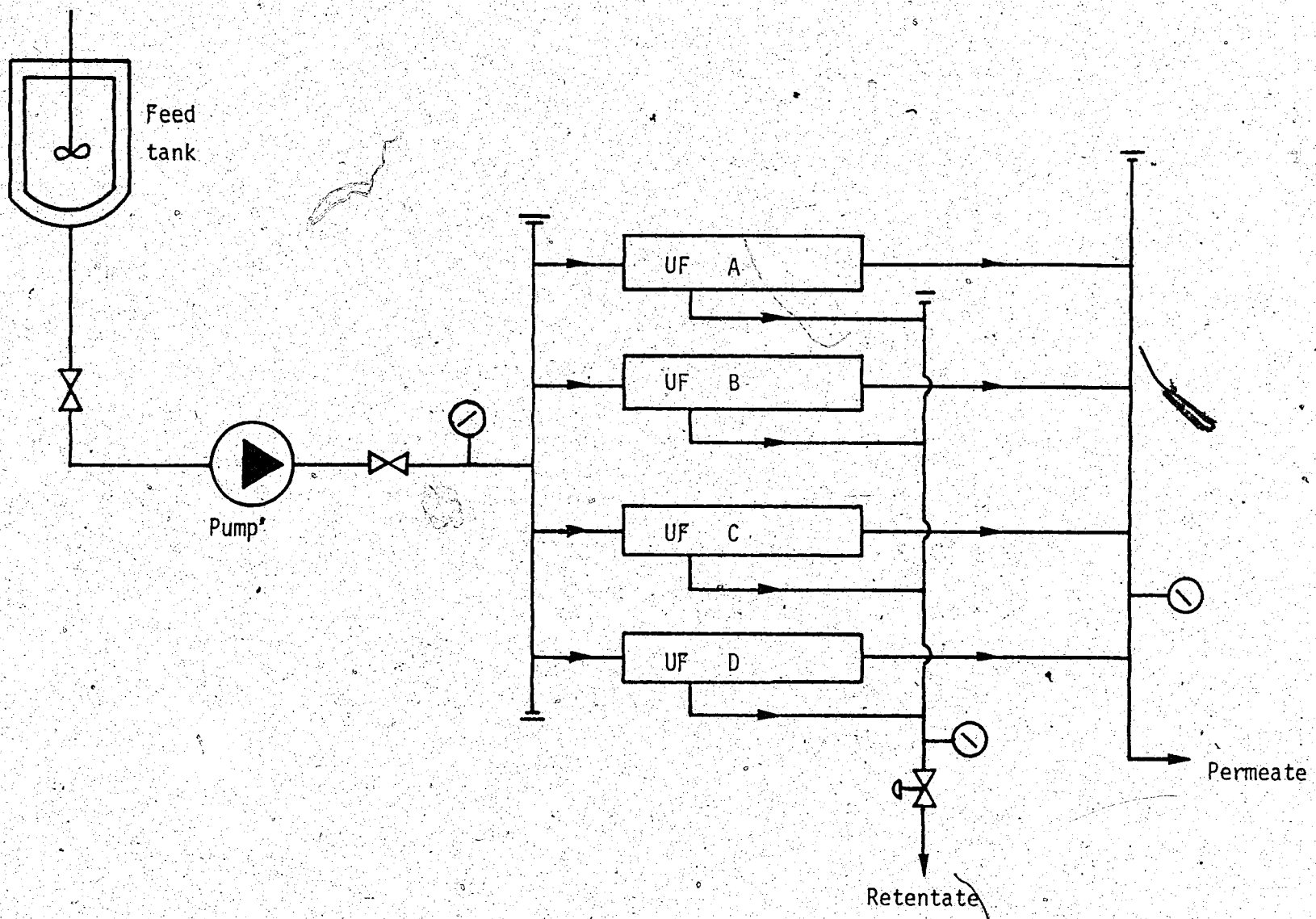


Figure 6. Diagram of an ultrafiltration process in a multi-stage parallel configuration. (Adapted from copper, 1981.)

operational configuration employed in this research was a single-stage batch-recycle arrangement (Figure 7).

The plate and frame stack design used in this research was a system containing flat sheet membranes. The module which housed the membranes consisted of a series of spacer plates and membrane plates. The spacer plates formed thin channels for the flow of the feed solution. A membrane was placed on each side of a support plate which was perforated to allow the permeate to flow. Alternating between each support plate was a spacer plate. This combination of alternate spacer and membrane support plates was assembled as a stack and compressed to hold them together to form the module (See Figure 4).

Ultrafiltration is highly suitable for purification, concentration and, sometimes, fractionation of biological materials. Some of the uses of ultrafiltration are:

1. in the paint industry for the removal of contaminants in the recycling of electrophoretic paints,

2. in the food industry for immobilised enzyme applications, treatment of dairy wastes such as whey, clarification of fruit juices (Heatherbell, 1977), clarification and cold sterilisation of wines (Cooper, 1980), fractionation of cattle blood plasma resulting in a cell concentrate and serum fraction, etc.,

3. in the pharmaceutical and electronics industry for the production of ultra-pure, pyrogen-free water (Cooper, 1980),

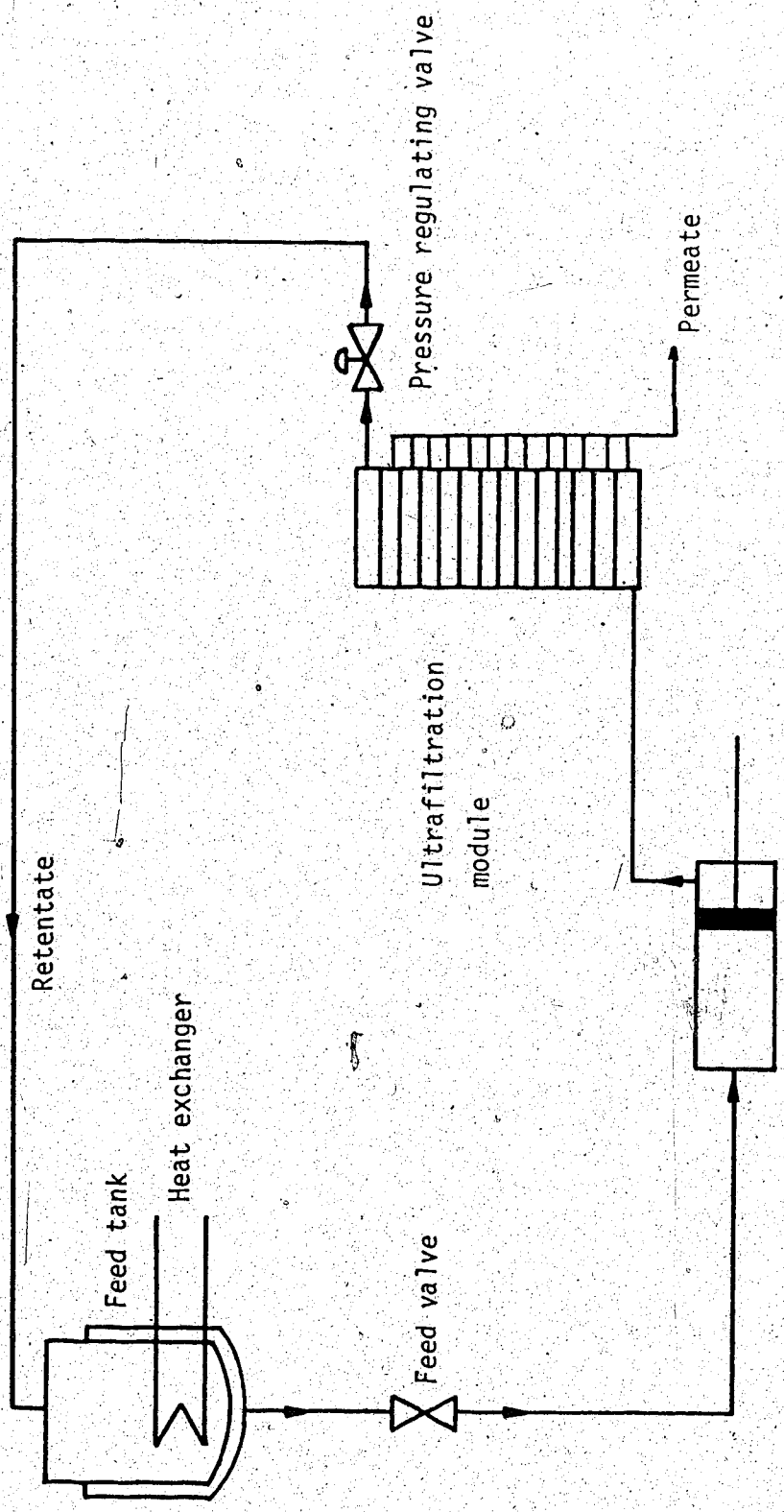


Figure 7. An ultrafiltration set-up in a single-stage batch-recycle configuration.

4. in the medical industries for the production of enzymes and medicinal drugs, and

5. in the paper industries for the treatment of waste water.

Ultrafiltration of diluted molasses has not been attempted. However, Harrison (1970) pointed out to the potential of this application in the sugar industry. Though ultrafiltration has been widely accepted as a unit operation in the various industries stated above, there remains major problems to be solved in the design of the equipment for efficient cleaning and prevention of membrane fouling due to precipitation on membrane surfaces. However, with rising energy costs and conservation, ultrafiltration will probably be more widely accepted as an energy efficient process for concentration as compared to 'traditional' methods e.g., evaporation.

3.3 Activated carbon

Activated carbon is an adsorbent made from materials such as lignite, nut shells, wood and coal (Mantell, 1968). Adsorbents are solids which are honeycombed with an infinite number of minute tunnels or pores. The dimensions of these pores are in the order of molecular sizes and are not of uniform size or shape. The distribution of the area within the pores of various sizes accounts for the specificity in adsorption. The carbons are activated by steam or chemicals (van Asbeck, 1981).

Activation involves the selective oxidation of the hydrocarbons on the surface of the carbon particles. The process of activation is also directed partly towards the development of a large area per unit weight of the adsorbent (McGinnis, 1951). The physical properties of the carbon, such as porosity, compactness and mechanical strength depends on the type and conditions of activation. The two most widely used adsorbents in the sugar manufacturing and refining processes are vegetable carbon and bone char.

Adsorbents are used for the decolourisation of sugars, vegetable oils, organic solutions, potable water, alcoholic beverages, air purification, odour removal, metals recovery, pharmaceutical and medicinal adsorbents for human ingestion. Carbon adsorbents are available in powdered, pelletised and granular forms. Commercial activated carbons on the basis of their physical structure and properties may be grouped into four classes namely decolourising, gas-adsorbent, metal-adsorbent and medicinal carbons. No one type of carbon can be universally used or is effective for all purposes (Mantell, 1968).

3.3.1 Types of activated carbon

The three main types of carbon adsorbents used in the sugar industry are bone char, granular and powdered carbons (Meade and Chen, 1977).

3.3.1.1 Bone char

Bone char or bone black is used in the decolourisation and refining of sugar. It is the carbonaceous residue obtained from the destructive distillation of bones. Bone char consists of a skeleton of calcium phosphate and carbonates with a very great number of minute tubes and channels. This skeleton is coated or lined with carbon in a fine state of subdivision and high activity (Meade and Chen, 1977). The adsorptive power of bone char resides in its activated carbon content.

Besides its decolourising power, bone char also adsorb ash constituents. The principal ionic constituents removed by bone char are calcium, magnesium and sulphate (Honig, 1953).

3.3.1.2 Granular carbon

Granular carbon or granular activated carbon is a high carbon adsorbent generally manufactured from coal. It contains more than 60% carbon and is steam activated (Mantell, 1968). Granular carbon in sugar refining has come into increasing use since the 1950's as a replacement or supplement to bone char. The chief advantage of granular carbon is its very high decolourising capacity; the chief disadvantage is its inability to remove ash, necessi tates an auxilary step to remove the ash after decolourisation. Granular carbon systems costs about 2½ to 3 times more in capital investment than

powdered carbon system but has the advantage of a considerably lower operating cost (Heroes, 1974, 1977).

3.3.1.3 Powdered activated carbon

Powdered activated carbon is carbon in a very fine state of division. It is usually prepared from the grinding of activated granular carbon. Coal and various other carbonaceous materials are used in the manufacture of powdered carbon. The performance of powdered carbon is approximately the same as granular carbon from the point of view of the amount of carbon required per unit sugar to achieve a certain amount or degree of colour removal. The two distinct advantages of powdered carbon are: the amount of contact time required for equivalent degree of decolourisation is only minutes as compared to hours for granular carbon; and it is much more effective in the removal of colloidal materials, giving better clarification of solutions. The disadvantage is that it is generally used a few times and discarded while bone char can be reused several hundred of times and granular carbon about 25 times (Meade and Chen, 1977).

Powdered carbon decolourisation is basically a batch process. The carbon is dispersed and kept suspended in the solution for a predetermined length of time before it is separated from the mixture by filtration.

3.3.2 Theory of carbon adsorption

Adsorption is a unit operation which deals with the utilisation of surface forces and the concentration of materials on the surfaces of solid bodies referred as adsorbents. Adsorption may occur alone, or may also be accompanied by chemical reaction the process of which is called 'chemisorption' (Mantell, 1951).

The theory of adsorption by an adsorbent involves many processes. It is necessary that the adsorbent and solution be in intimate contact for some time. The adsorbed atoms or molecules may be bound to the surface of the adsorbent weakly or strongly. The former is a physical process, also known as van der Waals adsorption. Most adsorption of sugar colourants occurs through this process (Mantell, 1951). The latter is termed 'chemisorption' and requires energy much the same way as chemical reactions. Adsorption is a function of temperature, the adsorbate, pressure, the adsorbing solid or adsorbent, its activity, origin and method of preparation (Mantell, 1968).

Several theories have been proposed to explain the mechanism of adsorption (Mantell, 1951):

1. Langmiur in 1915 proposed that adsorption is a type of chemical combination and the adsorbed layer is unimolecular.

2. Polyani in 1915 stated that adsorption is a physical process, electrical in nature, and the adsorbed layer is multimolecular. This theory is also known as Potential Theory

3. Zsigmondy in 1911 and Patrick in 1920 stated that adsorption is of physical nature, caused by capillary condensation.

4. Deboer, Zwikker and Bradley in 1929 proposed the Polarisation Theory which stated that when non-polar molecules are taken up on ionic adsorbents the assumption is that the adsorbent in its outermost layer induces dipoles in the first layer of molecules which are adsorbed. These in turn induce dipoles in adjacent layers and thus, by electrical attractive forces, several layers are built.

5. Brunauer, Emmett and Teller (also known as the B.E.T. Theory) stated that the adsorption phenomenon is a multilayer adsorption process.

However, despite these theories the exact mechanism of the adsorption process is still not fully understood. The B.E.T. Theory is currently the most widely accepted one (Mantell, 1951).

Adsorption involves the distribution of a substance between two phases, i.e. a liquid and a solid phase.

$$\text{Liquid} \rightleftharpoons \text{Adsorbate} \rightleftharpoons \text{Solid}$$

When a liquid containing impurities is brought into contact with a carbon, the attraction of the carbon for the impurities is greater than that of the liquid for the impurities. In this manner the equilibrium favours the removal of the

impurities from the solution by the adsorbent.

Sugar colourants are a mixture of many different compounds. Thus, any theoretical treatment is so complex that practical solutions to the adsorption theories have never been achieved (Meade and Chen, 1977).

3.3.3 Freundlich equation.

The Freundlich equation is also known as the Exponential equation. When a solution containing impurities is brought into intimate contact with a quantity of activated carbon, the removal of impurities from the solution by the carbon is very rapid during the first interval of contact and gradually reaches a point where increased time of contact gives no further decolourisation. If the relation between the adsorbed quantity of impurities and that present in the solution at equilibrium is studied, and the quantity of impurities removed from the solution or the amount adsorbed on the adsorbent is plotted against the concentration of the impurities remaining in the solution at equilibrium, curves concave to the concentration axis are obtained (Mantell, 1951). These curves (Figure 8) can be described by an exponential expression known as the Freundlich equation:

$$\frac{X}{M} = KC^{\frac{1}{n}}$$

where X = the weight of impurities adsorbed by M grams of adsorbent.

M = the mass of the adsorbent used.

C = the concentration of impurity remaining in the solution at equilibrium.

K and $\frac{1}{n}$ = constants which depends on the carbon used and the nature of the impurity.

Adsorption by decolourising carbons follows this exponential equation. Within the range of commercial practice this equation holds generally. Logarithmic plot of the exponential equation will produce a straight line (Figure 9). If the system is kept at constant temperature the straight line plot is called an adsorption isotherm. Under similar conditions, the decolourising ability of carbon increases as $\frac{1}{n}$ approaches unity (Mantell, 1951).

3.3.4 Designs of activated carbon decolourisation systems in sugar manufacturing and refining.

Due to operating costs and conditions, most activated carbon systems used in the sugar industries today are of the granular type. Granular bone char or activated vegetable carbons are the two most widely used adsorbents in the sugar manufacturing and refining processes.

Bone char and vegetable carbons are used in char filters. These are steel plate cisterns with inlets and outlets for passing feed and connecting attachments or devices for loading and discharging of the used or 'spent' adsorbent. Each filtering cycle consists of the following operation:

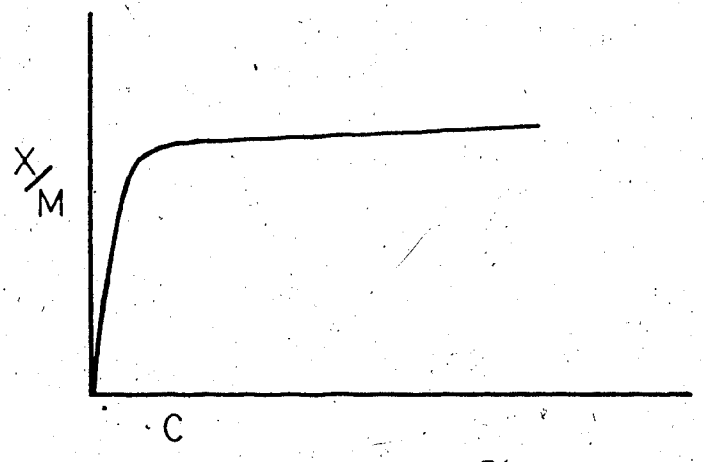


Figure 8. Decolourisation isotherm. [X/M (colour units removed per gram of carbon) versus C (residual colour concentration)] (From Mantell, 1951.)

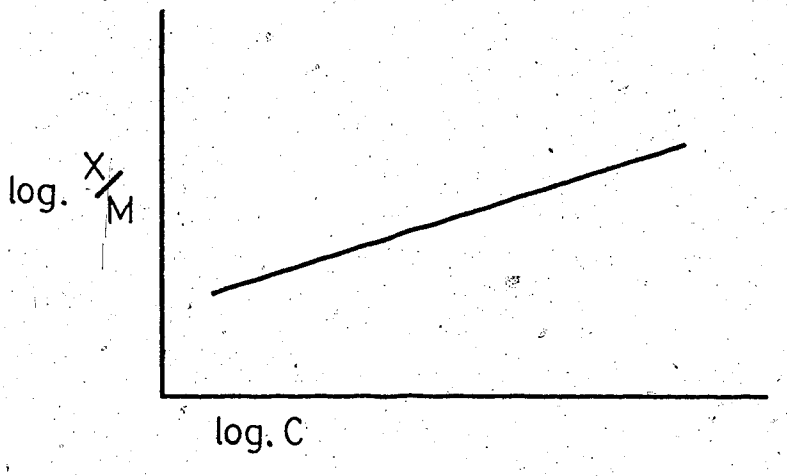


Figure 9. Decolourisation isotherm. [Log. X/M (Log. of colour units removed per gram of carbon) versus Log. C (Log. of residual colour concentration)] (From Mantell, 1951.)

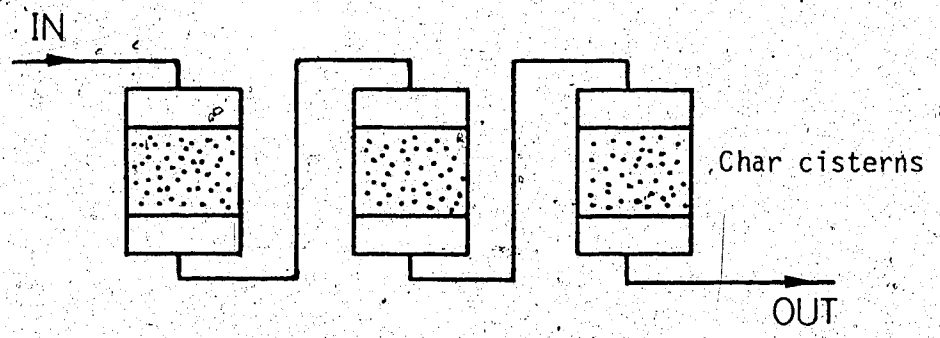
1. filling with the adsorbent (loading)
2. covering the adsorbent with liquor and settling the filter (sweetening-on)
3. running of the liquor or exhaustion of the filter bed
4. washing the sugar out of the adsorbent with hot water (sweetening-off)
5. washing with water which is sent to the waste to remove reversibly adsorbed material
6. blowing out the residual water with air
7. discharging the used adsorbent (dropping the filter)
8. regeneration or revivification of the adsorbent.

The two important aspects in the utilization of granular activated carbons in column operations are decolourising capacity and filterability. The operation can be conveniently divided into two systems (Mantell, 1968):

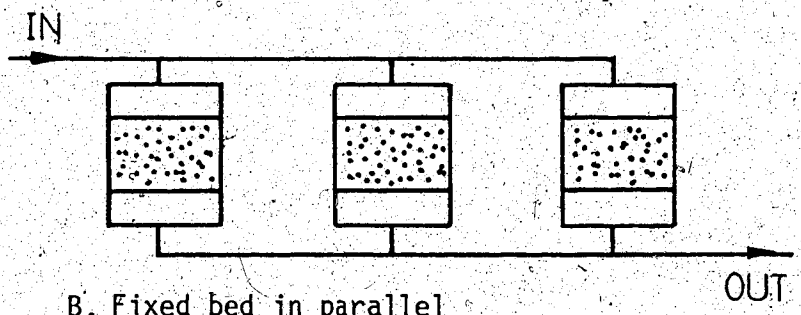
1. fixed-bed operations in which the sugar liquor flows through a stationary bed of adsorbent. This operation can be either in series or parallel (Figure 10, A, B) and

2. moving-bed operation in which both the liquor and adsorbent move countercurrently (Figure 10, C)

In fixed-bed operations with bone char or activated granular carbons, the liquor is passed through the adsorbent for a period that exhausts the adsorbent to a specific extent. The point at which the adsorbent is considered exhausted depends on a careful economic balance determined on the basis of each specific application.



A. Fixed bed in series



B. Fixed bed in parallel

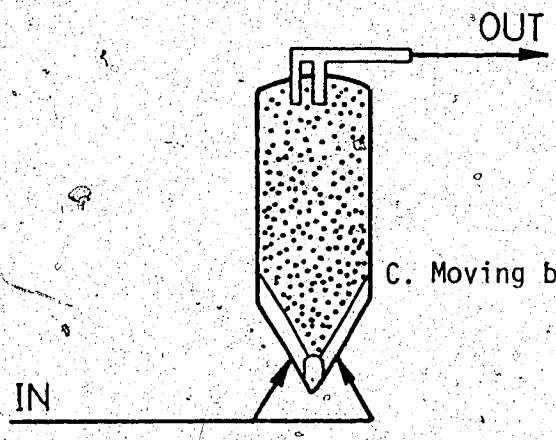


Figure 10. Configurations of char cisterns used in the sugar manufacturing process.
(From Mantell, 1968.)

3.4 Ion exchange resins

Ion exchange is a process in which an interchange of ions of the same polarity occurs between a solution and an essentially insoluble solid in contact with the solution (Skoog, 1969). Many substances, both natural and synthetic, have been used as ion exchangers. Common clays, celluloses, proteins and zeolites (natural sodium aluminate complexes) are natural ion exchange materials that have been studied. However, these natural substances have low exchange capacities and other unfavourable chemical and physical properties (Khym, 1974). Synthetic organic ion exchange resins were first produced in 1935 and have since had widespread laboratory and industrial applications e.g. for water softening, water deionisation, solution purification and ion separation.

Synthetic organic resins are solids consisting of two parts: the basic framework which is an elastic, three-dimensional hydrocarbon network, and the ionisable or functional groups attached to this organic matrix. These functional groups have active (mobile) ions that can react or be replaced by other ions (Khym, 1974).

The chemical behaviour of ion-exchange resins is determined by the nature of the functional groups attached to the hydrocarbon skeleton. The two major classes of ion exchange polymers are cation and anion exchangers.

Cation exchangers may be strong cationic or acid resins

containing sulphonic acid groups (RSO_3H) (Figure 11, A) or weak acid resins containing carboxylic groups (RCOOH) (Figure 11, B). Anion exchange resins contain amine functional groups attached to the polymer. Strong base exchangers are quarternary amines $\left[RN(CH_3)_3^+ OH^-\right]$ and weak base types contain secondary and tertiary amines (Figure 12).

Ion exchange is particularly effective when the concentrations of impurities to be removed are very low (Kunin and Pollio, 1968). The capacity of ion exchange resins are expressed in milliequivalents. The number of exchange sites on the resin matrix determines the exchange capacity of the ion exchanger for counter-ions. This capacity is usually expressed on a dry-weight or wet-volume basis. The dry-weight capacity is the number of exchange sites in milliequivalent per unit dry weight of resin and the wet-weight capacity is the number of exchange sites in milliequivalent per unit volume of resin swollen in water.

Chemical and thermal degradations of the matrix material are the chief causes of resin deterioration. Stability of resin varies with resin type, operating temperature, ionic form of the resin and the operating pH (Kunin and Pollio, 1968).

3.4.1 Demineralisation

Demineralisation or deionisation is the removal of cationic and anionic impurities from a solution. Complete

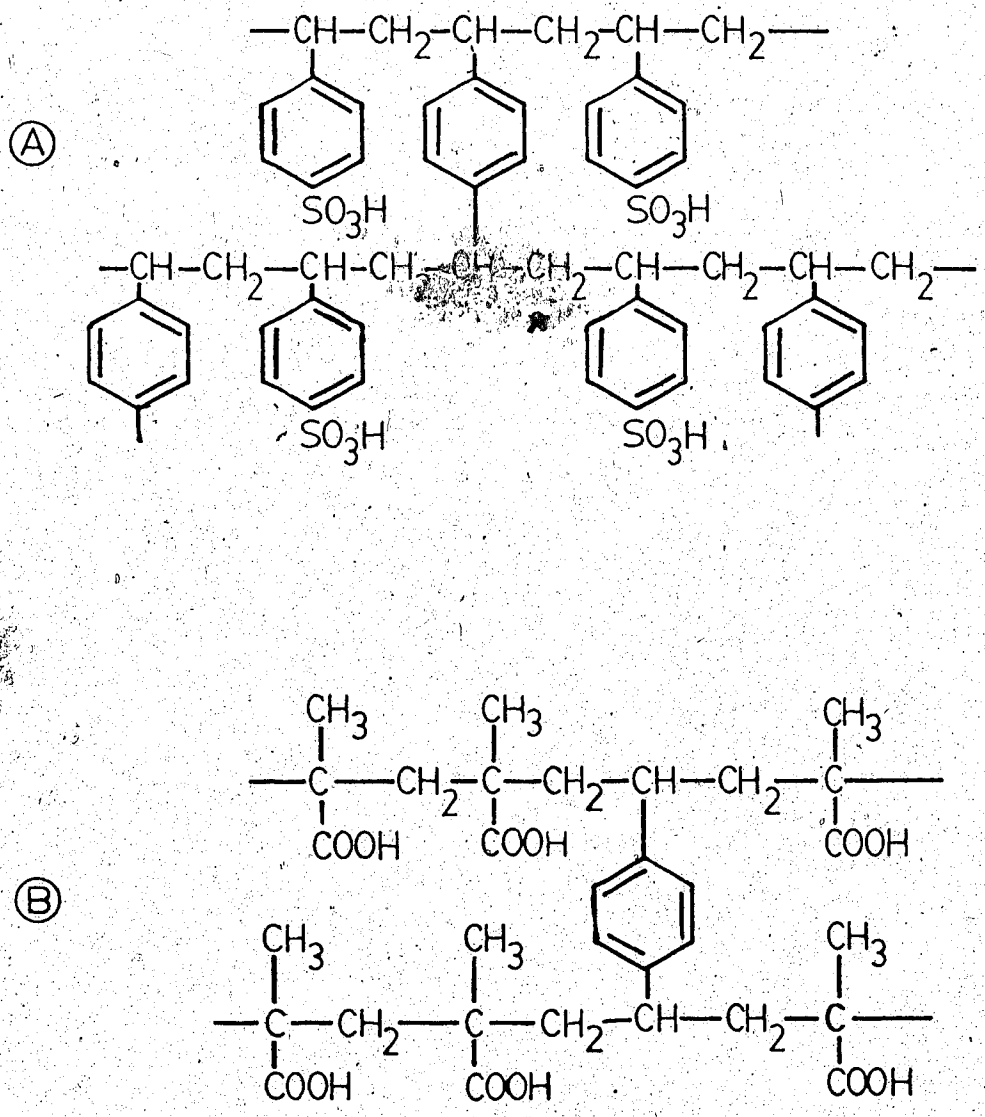


Figure 11. Chemical structures of cation exchange resins.
Ⓐ Strong-acid polystyrene type cation exchange resin.
Ⓑ Weak-acid acrylic type cation exchange resin.

(From Khym, 1974.)

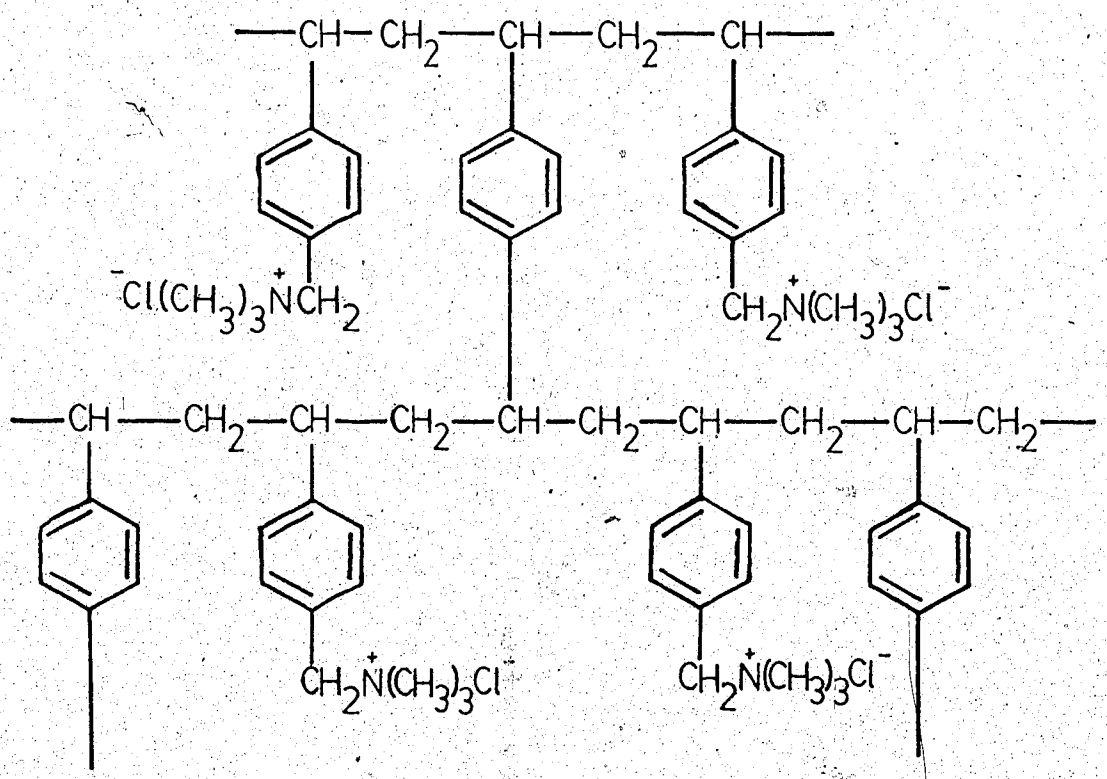


Figure 12. Chemical structure of a strong-base ammonium polystyrene type anion exchange resin. (From Khym, 1974.)

removal of ionic impurities from a solution can be achieved through ion exchange using the hydrogen form (H^+) of cation and the hydroxide form (OH^-) of anion exchange resin.

There are three basic methods for employing ion exchange resins in deionisation process. The first, conventional deionisation, involves passage of the solution through a column of a cation exchanger (H^+ form) to form free acids from the ions present and then through the basic form of an anion exchanger to remove the acids. The second, reverse deionisation, is similar to the first except that the solution is first passed through the anion exchanger to form bases from the ions present and then through the cation exchanger to remove the bases. The third, mono-bed or mixed-bed deionisation, involves contacting the solution batchwise or columnwise to a mixture of cation and anion exchangers in the H^+ and OH^- forms, respectively.

Various combinations of weak or strong cation or anion exchange resins may be employed. The conventional and reverse deionisation systems can give rise to two undesirable effects:

1. The formation of a temporary acidic or basic condition.
2. The existence of a reversible exchange equilibrium during the first stage of the deionisation.

For many applications, a temporary acidic or basic condition can drastically alter the properties of a substance. This can be particularly critical for the deionisation of biochemical solutions containing proteins, sugars etc. In the

sugar industry this can lead to losses of sucrose through inversion and/or colour formation.

Realisation of these deficiencies led to the development of the mono-bed or mixed-bed technique which is able to maintain a neutral medium and allows complete deionisation. The mono-bed technique has become well accepted and is being used world wide. Combinations involving strong acid cation exchange resins with strong base anion exchange resins are routinely used to produce water of very high quality (Khym, 1974).

3.4.2 Ion exchange techniques

Ion exchange applications may be carried out by either column or batch techniques. Column operations are usually preferable although the choice of method depends entirely upon the application in question.

In column operations the ion exchange resin is placed in a vertical column to form a bed. The solution to be treated is passed through this column until a predetermined endpoint is reached and the resin may then be regenerated to be used in another cycle.

In batch operations the ion exchange resin is simply agitated in a vessel together with a given quantity of the solution to be treated. When a predetermined endpoint is reached, the solution is removed and the resin regenerated.

3.4.3 Demineralisation as applied to sugar industry

Demineralisation with synthetic organic resins as a unit operation in sugar industry have been attempted since the middle 1940's (Hoareaus *et al.*, 1977). Sugar industry was one of the first to use ion exchange resins in its manufacturing processes. When acid and base stable resins were developed, attempts were initiated to improve the recovery of sucrose by deionisation of beet sugar juice.

Difficulties in resin stability and sucrose inversion slowed these developments for several years until new and better resins were developed. Demineralisation was used to achieve two goals: the removal of melassigenic salts to increase sucrose recovery, and the decalcification of sugar juices to reduce the formation of scales on heat exchange surfaces (Pollio and McGarvey, 1977).

Demineralisation of the thin juices is carried out on a strong acidic cation exchanger made of sulphonated polystyrene and then on an anion exchanger which is generally weakly basic (Pollio and McGarvey, 1977). In order to avoid the inversion of the sucrose the treatment is performed at a temperature of lower than 12° C. Demineralisation increases sugar extraction to 88 - 89%, whereas without demineralisation the rate is only 82 - 86%, depending on the operation (Nachod, 1949).

The primary purpose of demineralising sugar solutions

is to remove impurities, both inorganic and organic. The benefits of the increase in purity of the treated solutions are manifested in numerous ways depending on the type of sugar solution being processed and the products obtained. According to Nachod (1949) application of demineralisation step in sucrose manufacture from both beet and cane results in

1. greater yields of sucrose as purer liquor goes into sugar boiling pans,
2. subsequent decrease in molasses formation,
3. improvement of sugar and final molasses quality,
4. elimination of evaporator scaling and intermittent washouts of juice heaters and evaporators,
5. superior working qualities of the massecuite, and
6. some saving in chemicals in beet juice processing.

3.5 Hydrolysis (inversion) of sucrose

Hydrolysis or inversion is the splitting of the glycosidic bond between the monosaccharides glucose and fructose of sucrose. The hydrolysis of sucrose theoretically produces a 50:50 mixture of D-glucose (dextrose) and D-fructose (laevulose). If the hydrolysis is not carried out to completion some sucrose can remain in the product. The word 'inversion' is applied because the original solution of sucrose, when polarised, gives a right hand rotation (dextrorotatory) to the plane of

polarised light, while the final solution of invert sugar gives a left hand rotation (laevorotatory) (McGinnis, 1951). Three methods have been used in the manufacture of invert sugar. These include the use of:

1. strong mineral or weak organic acids,
2. the enzyme invertase, and
3. ion exchange resins.

3.5.1 Hydrolysis with strong or weak acids

In the presence of hydrogen ions a hydrolytic decomposition of dissolved sucrose takes place. The extent of the inversion depends on the nature of the acid (mineral acids have a much greater inverting power than organic acids), its quantity in relation to the amount of sucrose, the temperature of the acid-sucrose mixture (the higher the temperature the greater the inversion) and the length of time the acid and sucrose are in contact (Meade and Chen, 1977). In dilute solutions the rate is directly proportional to both sucrose and hydrogen-ion concentrations. The temperature coefficient of the reaction (Q_{10}) is about 2.8 (Honig, 1953). Sugar inversion is exothermal and the heat generated which is a function of the reaction time is directly related to the percent of invert produced (Honig, 1953).

Alkali has to be added to neutralise the acid when the desired degree of inversion or hydrolysis has been reached. The addition of substantial amounts of such alkali has the

effect of increasing the inorganic content of the product, contributing to colour development and decomposition of fructose at high pH (Hughes *et al.*, 1952).

3.5.2 Hydrolysis with invertase

Invertase is known by many names such as saccharase, α-glucosidase, β-D-fructofuranosidase, β-D-fructosidase, and β-D-fructofuranoside fructohydrolase. The Enzyme Commission designated number for this enzyme is E. C. 3.2.1.26 (Chibata, 1978). Invertase of commercial importance is obtained from cultures of yeast. The word invertase encompasses a whole family of sucrose-hydrolysing enzymes. Only Saccharomyces cerevisiae and Saccharomyces carlsbergensis are important in the industrial production of invertase (Reed, 1966).

Invertase is used extensively in the confection manufacturing industry. It finds very little application in the sugar manufacturing industry. In the sweetener industry, it is used for the continuous production of invert sugar from sucrose. This enzyme has been immobilised in many fashions onto various support materials. For example, it has been immobilised by the diazo method using porous glass, by ionic bonding method using DEAE-cellulose, and entrapping methods using polyacrylamide gel and cellulose acetate (Chibata, 1978). The first practical proposal to use immobilised enzymes in an industrial process involved invertase. Tate and Lyle were issued a patent for the

simultaneous inversion and decolourisation of sugar liquor by passing it through invertase adsorbed on bone char (Schwimmer, 1981).

Due to excessive competition from the corn syrup industry employing immobilised glucose isomerase in their production of HFS, invertase is no longer of economical importance. It is now used mainly for analytical purposes (Schwimmer, 1981).

Enzymatic inversion employs a more 'gentle' condition for hydrolysis of sucrose which gives a cleaner product with less by-products and hence requiring less post treatment or refining. The main drawbacks are the high operating costs and the cost of the enzyme itself.

3.5.3 Hydrolysis with ion exchange resins

The hydrolysis of sucrose can be carried out very simply on a cation exchanger resin in the free acid or H^+ form. The sucrose solution is percolated through a strong acid exchanger in a heated column. The correct percentage of inversion is obtained by varying the temperature or the percolation speed. The regeneration of the resins is carried out with any dilute mineral acids, most preferably HCl or H_2SO_4 .

Ion exchange resins used usually have a divinylbenzene percentage or content less than conventional resins so as to facilitate the penetration of the resin beads by the solute

(Hughes *et al.*, 1952). Figure 13 shows a flow chart of invert sugar production by ion exchange resin as outlined by Hughes (1952).

Ion exchange requires continuous operation and yields a high quality product. Initial capital costs and operating costs, i.e. regenerating chemicals, resin makeup, sugar loss, dilution, cooling, evaporation and waste regenerant chemical disposal are high.

3.6 Separation of monosaccharides

The separation of glucose from fructose involves the formation of complexes between these monosaccharides and alkaline earth metals or their hydroxides (Moulik and Mitra, 1973). An unstable chemical addition compound called an adduct is formed (Roy and Mitra, 1972). However, the field of sugar-metal complexes is still largely unexplored (Angyal, 1973).

In the beet sugar industry the sugar-metal complexing phenomenon has been used in the Steffan Process for recovery of the sucrose in molasses (McGinnis, 1951). Adducts are formed in a stoichiometric ratio of 1 part carbohydrate to 1 part of metal ion. Experiments to study the yield of adduct in relation to different molar proportions of sugar have established that the yield is highest when the components are mixed in 1:1 molar proportion (Roy and Mitra, 1972). Metal ions studied in adduct formation are calcium, strontium, lithium, barium and silver (Moulik and Mitra, 1973). Moulik and Khan (1975) also found

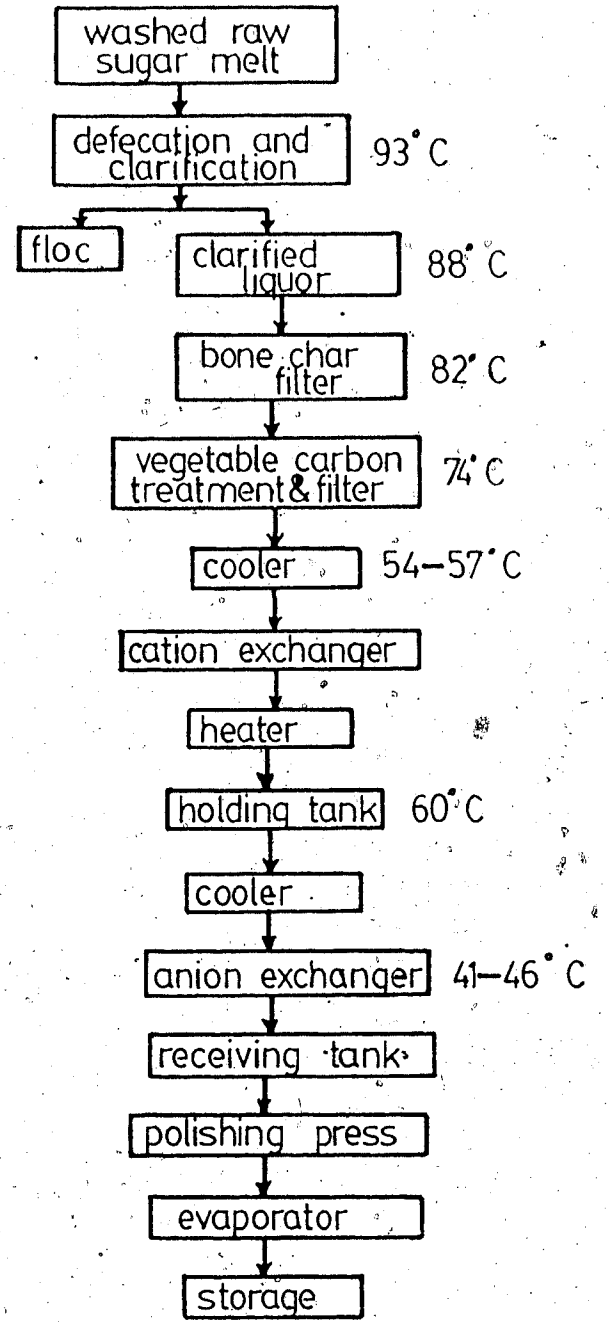


Figure 13. Flow chart of invert syrup process using strong cationic exchange resin. (From Hughes *et al.*, 1952.)

that reducing sugars bind more strongly to metal ions than non-reducing sugars.

The binding process in adduct formation occurs between hydroxyl groups of the carbohydrates with metal ion (Moulik and Mitra, 1973). The metal ion used can be from a soluble salt (e.g. chloride) or a hydroxide. It can be 'free' in solution or fixed on an ion exchange resin or silica gel (Briggs *et al.*, 1981). This complex formation makes possible the separation of some sugars on ion exchange columns.

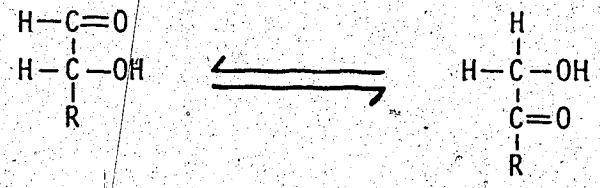
Commercial corn syrup contains 42% fructose, 50% dextrose and 8% oligosaccharides. However, when the desired content of fructose in the syrup is greater than 42% separation of glucose from fructose becomes a necessary step in the process. There have been several patents on methods for the separation of these sugars by ion exchange resins (Samuelson, 1953 and Petersen, 1975). The resins are not utilised in these systems as ion exchangers but rather as chromatographic adsorbents (Petersen, 1975). Monosaccharides dissolved in ethanol are adsorbed on an anion exchange resin (HSO_3^- form) (Samuelson, 1953). Elution with ethanol solutions of gradually reduced concentration leads to the separation of the sugars. Other methods involve subjecting a syrup containing fructose and glucose to a column containing a sulphonated polystyrene cation exchange resin charged with an alkaline earth metal e.g. Ca^{2+} or Ba^{2+}. The eluant is water. Feeding and elution is sequential

by admitting predetermined volumes of the syrup and water to the column. Fructose is preferentially adsorbed by the resin and is subsequently eluted after glucose. With a system of valves, various fractions can be collected. Some of the fractions are recycled to achieve the desired degree of separation, or fructose content. Feed rates and operating temperature are important parameters controlling the efficiency of the separation (Petersen, 1975).

3.7 Isomerisation

3.7.1 Isomerisation of sugar

Isomerisation of sugar is defined as a rearrangement or an intramolecular transformation of hydrogen atoms between adjacent carbon atoms in a sugar molecule (Casey, 1977). This mechanism may be illustrated as follows:



Aldose (e.g. glucose) Ketose (e.g. fructose)

In the commercial process isomerisation of glucose to fructose reaches an equilibrium at approximately 1:1 ratio of glucose to fructose. Isomerisation of sugar can be catalysed by alkaline conditions or by enzymes (Figure 14).

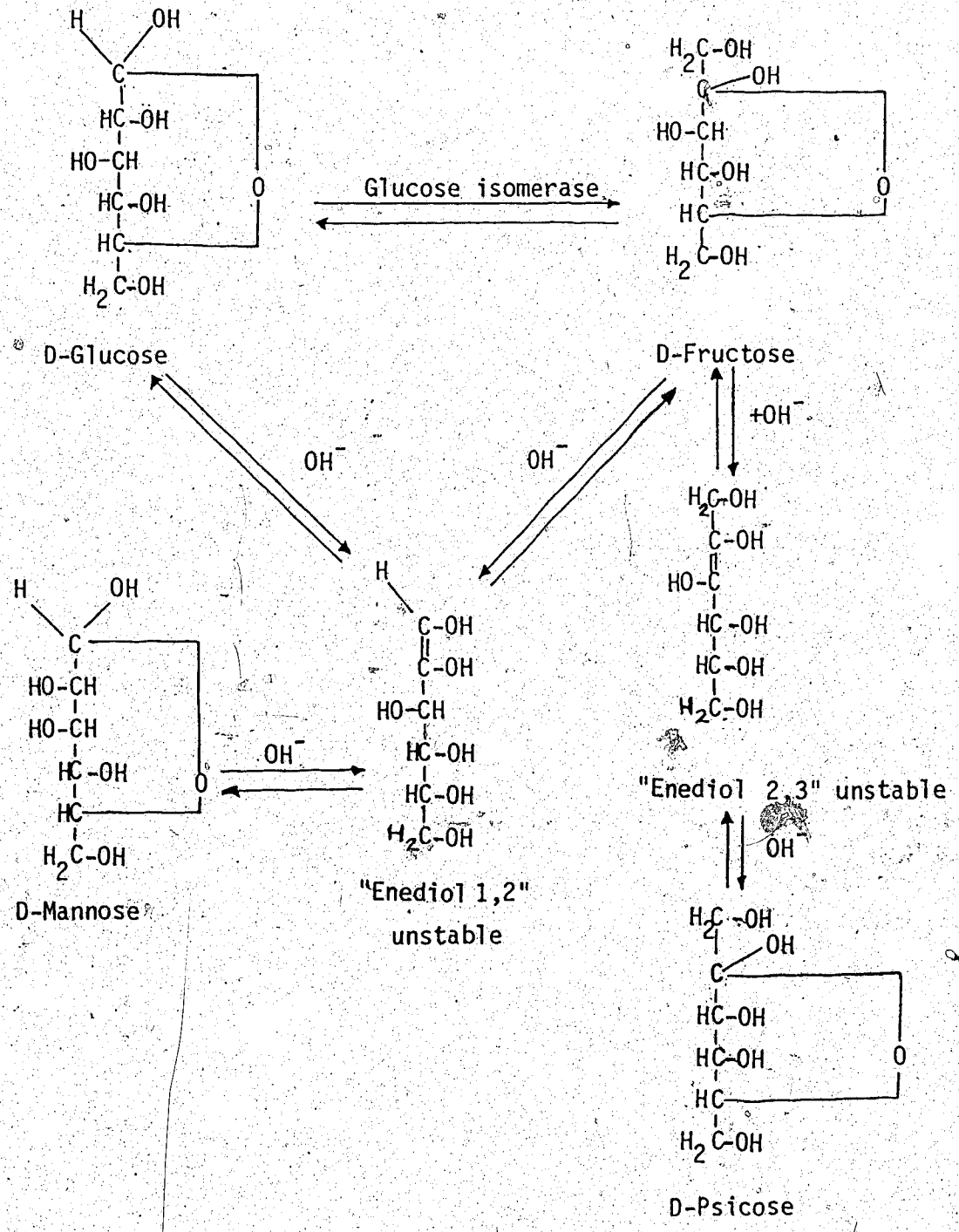


Figure 14. Enzymatic and alkaline isomerisation of glucose to fructose
(From Geyer, 1974.)

3.7.2 Isomerisation of sugar with enzymes

Glucose isomerase catalyses the isomerisation of glucose to fructose. This enzyme is also known as D-xylose ketol-isomerase and is designated by the Enzyme Commission as E. C. 5.3.1.5. The discovery of ketol isomerase revolutionised the sweetener industry making the commercial production of HFS possible. The underlying principles of sugar isomerisation are rooted in the investigations of the biochemistry of the metabolism of carbohydrates within bacterial and animal cells. Complex carbohydrates are first broken down to glucose which is then phosphorylated to glucose-6-phosphate. An isomerase then transforms glucose-6-phosphate into an equilibrium mixture of glucose-6-phosphate and fructose-6-phosphate (Casey, 1977).

Much of the work on the utilisation and commercialisation of glucose isomerase is credited to a Japanese research team working at the Japan Institute of Fermentation (Casey, 1977). These investigators studied the effect of temperature, pH, metal ions, concentration of substrate and oxygen on the kinetics and equilibrium reactions of this enzyme.

Commercialisation of the technology based on the scientific finding became feasible when Takasaki (1966) discovered a cheaper method for the production of glucose isomerase. He found that pure xylose which had been used as the

sole source of carbon for glucose isomerase producing organisms could be substituted with a much cheaper xylan. A wide variety of microorganisms has been used in commercial preparation of the enzyme. They include Lactobacillus, Pseudomonas, Actinoplanes, Pasteurella, Leuconostoc, Streptomyces and Aerobacter (Johnson, 1976).

The enzyme can be employed in the soluble or insoluble (commonly referred to as immobilised) form. Economics and operating considerations strongly favour the immobilised form. Major corn syrup producers use immobilised glucose isomerase in tall columns called reactors. These reactors can be operated in series, parallel or moving-bed configuration similar to granular carbon systems (See Figure 10). Commercial immobilised glucose isomerase is generally available in two forms: a granular and a fibrous or amorphous form. Table 2 lists some of the major processors/distributors of HFCS and glucose isomerase (Schwimmer, 1981).

Glucose isomerase has been called the 'Cinderella' of all industrial enzyme processes due to its significance in the food sweetener industry (Barker, 1978). This process of isomerisation could cause a revolution in the pattern of world agriculture, since it is now feasible for each country to grow cereal or root crops, which provide the most economic source of starch, to be converted into its own indigenous source of sweetener to replace much of its sucrose requirement (Gramera, 1978).

Table 2. Some processors/distributors of HFCS and glucose isomerase

Trade name	Holding company and licensee or *subsidiary*	Source of enzyme
Amerose	Amstar	———
Corn Sweet	ADM	———
Invertose	CPC	Streptomyces chromogenes
Isomerose	Standard Clinton	Streptomyces alba
Isosweet	Staley	Streptomyces spp.
Iso-syrup	Miles	Streptomyces olivaceus
Maxazyme	Anheuser-Busch Gist-Brocade	Actinoplanes missourensis
Sweetzyme	Novo	Bacillus coagulans
TruSweet	American Maize	———
Various	Reynolds, KHS	Anthrobacter spp.

(From Schwimmer, 1981.)

3.7.3 Other methods of isomerisation

The possibility of converting glucose to fructose has been explored for over 150 years since the classic investigations of Lobry de Bruyn and Alberda von Eckstein on the alkaline isomerisation of glucose (Schwimmer, 1981). It has since been known that glucose can be isomerised to fructose by treating it with alkaline catalysts at high pH.

Many commercial patents have been issued for the isomerisation of D-glucose to D-fructose. Isomerisation have been performed using cyclohexylamine, alkaline solution containing aryl boric acids and macroreticular basic resins. The amount of fructose obtained by resin isomerisation depends on a number of factors, such as resin contact time, temperature, solids level of the feed, type of resin, method of resin regeneration, etc. (Johnson, 1976). With alkaline isomerisation, however, it is difficult to attain more than 40% fructose without forming accumulated amounts of nondextrose and nonfructose degradation products which results in the reduction of yield (See Figure 14).

Figure 15 shows a process for the production of HFS by alkaline isomerisation of glucose with macroreticular basic resin (Johnson, 1976).

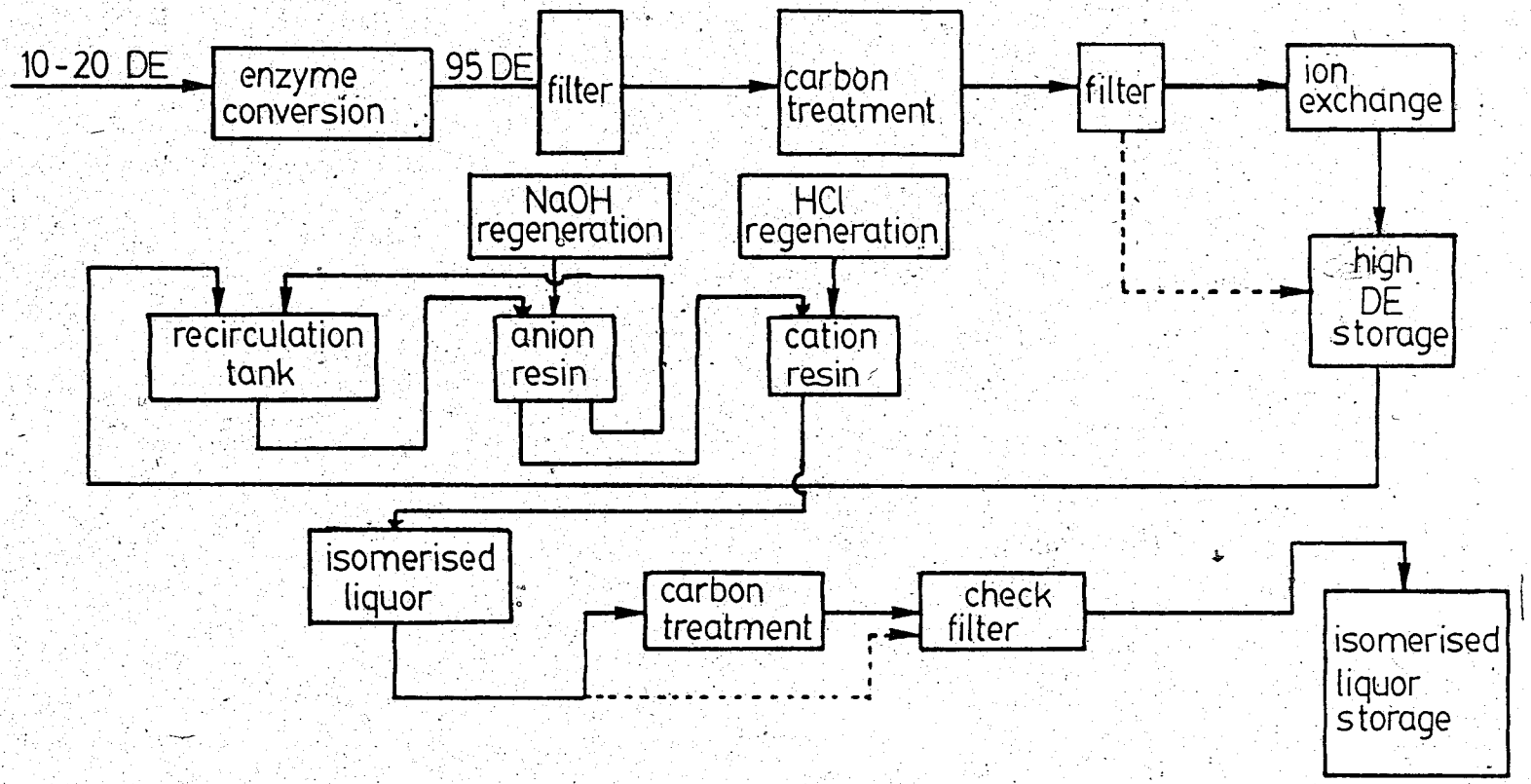


Figure 15. Flow diagram of alkaline isomerisation of glucose to fructose using a macroreticular basic resin.
(Adapted from Johnson, 1976.)

4. EXPERIMENTAL

4.1 Purification of molasses

4.1.1 Ultrafiltration

4.1.1.1 Material

Alberta final beet molasses of the 1981 campaign were obtained from Alberta Sugar Company, Lethbridge, Alta.

4.1.1.2 Equipment

De Danske Sukkerfabrikker plate and frame model LAB - 20 MODULE with 20 GR 8P membranes of 10,000 nominal molecular weight cut-off. Feed and operating pump was a high pressure reciprocating piston pump, De Danske Sukkerfabrikker DDS RO - Division, DK - 4900, Naskov, Denmark.

Braun Thermomix 1480 circulator, B. Braun, Melsungen, West Germany.

Milli-RO 60 water purification system, Millipore Corp., Bedford, MA.

4.1.1.3 Procedure

Approximately 3500 g (± 50 g) of beet molasses was made up to 10 L with Milli - RO water in a stainless steel container. The diluted molasses was analysed for pH, colour, ash and sugar content according to the procedures described in subsequent sections, warmed to about 50° C prior to being

ultrafiltrated.

The ultrafiltration module housing the membranes and support plates were assembled according to the DDS LAB MODULE operation manual No. 1694 - GB - 0678 - 50. Twenty GR 8P membranes together with 10 spacer and support plates were assembled giving a total membrane filtration area of 0.36 m^2.

The diluted and warmed beet molasses was ultrafiltrated with the DDS LAB MODULE according to the operation procedure outlined in section E of the operation manual under the following conditions:

- temperature 50° C
- pressure of ≈ 1000 kPa (≈ 145 psi)
- feed rate of 6 L/min

A volume concentration ratio of 2.50 (VCR - ratio of initial feed volume to final retentate volume) was performed before the operation was terminated.

The retentate and permeate were analysed for pH, total solids, colour, ash and sugar content.

4.1.2 Powdered activated carbon treatment

4.1.2.1 Material

Powdered activated decolourising carbon (neutral - Norit) was from Fisher Scientific Co., Fair Lawn, NJ.

4.1.2.2 Procedure

The permeate from ultrafiltration was decolourised with activated carbon in a batch stirred method according to procedure described by Mantell (1968) under the following conditions:

- a temperature of 65 to 70° C
- activated carbon dosage of 35% of total solids of the permeate
- contact time of 35 to 45 minutes.

The activated carbon treated product was then filtered through a Buchner funnel and the filtrate was analysed for pH, colour, ash and sugar content.

4.1.3 Demineralisation (Batch method)

4.1.3.1 Materials

Sucrose crystals ($C_{12}H_{22}O_{11}$) and Rexyn I - 300 mono-bed resin beads (See Appendix II for specifications) were obtained from Fisher Scientific Co., Fair Lawn, NJ.

4.1.3.2 Procedure

The filtrate from activated carbon treatment was partially demineralised in a batch stirred method by adding mono-bed resin beads at the amount of ¼ of the volume of the solution to be treated.

The resin mixture was initially 'sweetened-on' with pure sucrose solution of a concentration similar to the

activated carbon treated molasses.

The resin-filtrate mixture was stirred slowly for 15 to 20 minutes at ambient temperature before it was filtered through a Buchner funnel. The partially demineralised solution was analysed for pH, colour, ash and sugar content.

4.1.4 Demineralisation (Column method)

4.1.4.1 Materials

Sucrose crystals ($C_{12}H_{22}O_{11}$) and Rexyn I - 300 mono-bed resin beads were obtained from Fisher Scientific Co., Fair Lawn, NJ.

4.1.4.2 Equipment

Pharmacia jacketed chromatography column K 26/40 complete with flow adapters, reservoirs (R 15 and R 25) and 3-way valves, Pharmacia Canada Ltd., Dorval, P.Q.

ISCO fraction collector, type 550, Instrument Specialties Company Inc., Lincoln, NE.

4.1.4.3 Procedure

Approximately 100 mL of the mono-bed resin mixture was packed into the chromatography column and sweetened-on with sucrose solution according to the 'resin packing procedure' outlined in Appendix I. A Mariotte flask set-up (Figure 16) was used for maintaining the desired and uniform flow rate. Upflow or ascending chromatography was used.

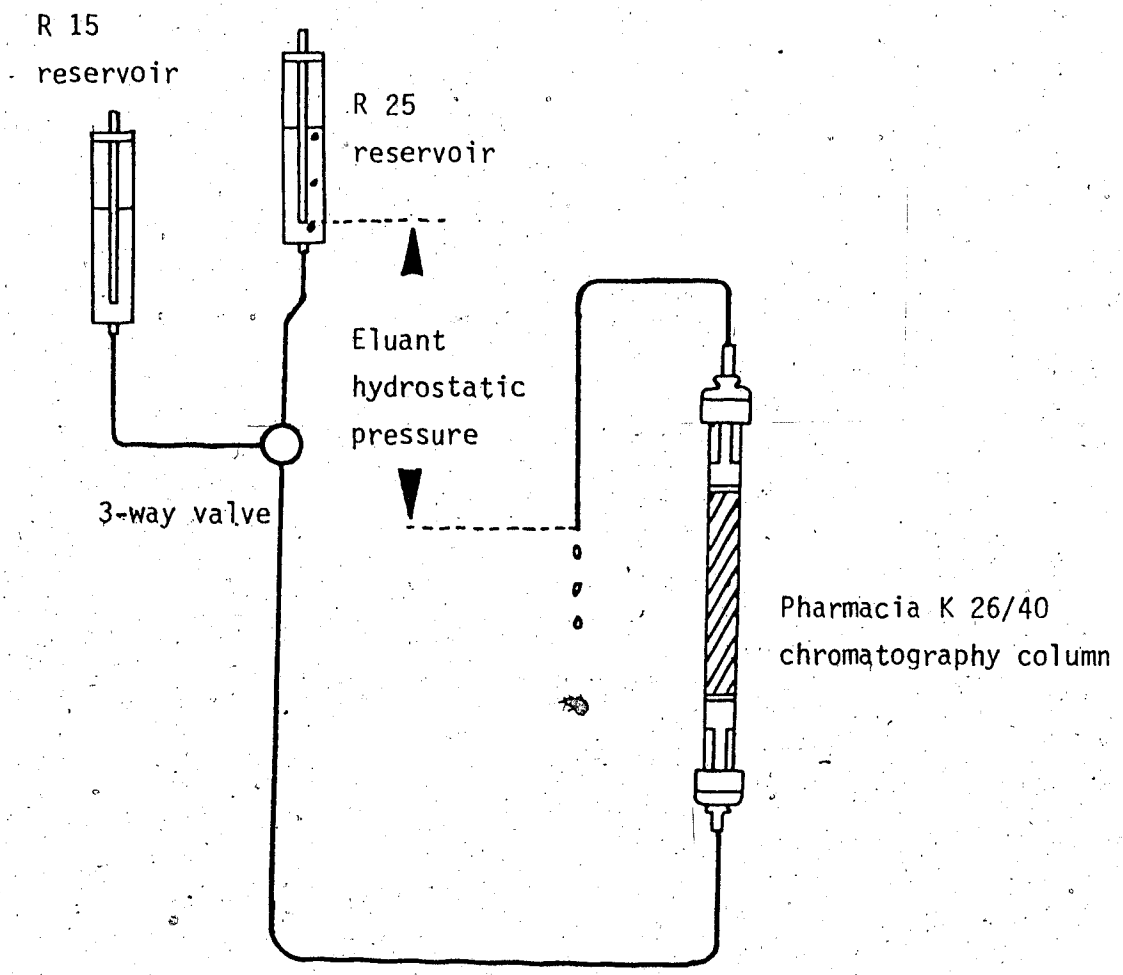


Figure 16. A typical Mariotte flask set-up for maintaining uniform flow rate.

When the resin bed had been properly equilibrated and sweetened-on , the activated carbon treated solution was fed through the bed at the flow rate of 0.88 mL/cm^2/min at ambient temperature to a total of 4 bed volumes before the operation was terminated.

Approximately 6 mL fractions were collected with a fraction collector and each fraction was analysed for ash content. The fractions were then mixed and analysed for pH, colour and sugar.

4.2 Production of high fructose syrup

4.2.1 Hydrolysis of sucrose.

4.2.1.1 Materials

Sucrose crystals ($C_{12}H_{22}O_{11}$), ACS grade, were obtained from Fisher Scientific Co., Fair Lawn, NJ.

Analytical grade cation exchange resin in H^+ form, AG 50W - X4, were obtained from Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont. (See Appendix II for specifications.)

4.2.1.2 Equipment

Pharmacia jacketed chromatography column K 26/40 and complete with flow adapters, reservoirs (R 15 and R 25) and 3-way valves, Pharmacia Canada Ltd., Dorval, P.Q.

Braun Thermomix 1441 circulator, B. Braun, Melsungen, West Germany.

4.2.1.3 Procedure

Approximately 100 mL of a strong cationic exchange resin (H^+ form) was introduced into the chromatography column and sweetened-on with sucrose solution according to the 'resin packing procedure' outlined in Appendix I. A Mariotte flask set-up with ascending chromatography as shown in Figure 16 was used to maintain the desired flow rate.

The hydrolysis operation was performed at 60° C with a feed flow rate of 0.19 mL/cm^2/min. A total of 4 bed volumes were treated before the operation was terminated.

The hydrolysate was analysed for pH, colour, and sugar content.

4.2.2 Separation of glucose and fructose

4.2.2.1 Material

Analytical grade cation exchange resin in Ca^{2+} form (See Appendix I for conversion process and Appendix II for specifications) were obtained from Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont.

4.2.2.2 Equipment

Pharmacia jacketed chromatography column K 26/40 complete with flow adapters, reservoirs (R 15 and R 25) and 3-way valves, Pharmacia Canada Ltd., Dorval, P.Q.

ISCO fraction collector, Type 550, Instrument Specialties Company Inc., Lincoln, NE.

Braun Thermomix 1441 circulator, B. Braun, Melsungen, West Germany.

4.2.2.3 Procedure

Approximately 100 mL of a cationic resin in Ca^{2+} form was charged into the chromatography column according to the 'resin packing procedure' as outlined in Appendix I. Again, a Mariotte flask set-up with ascending chromatography was used to maintain the flow rate.

The separation operation involved sequential feeding of feed followed by elution with distilled water according to Johnson (1976). Partial separation of the monosaccharide mixture was achieved under the following conditions: loading of feed at 10% of bed volume; operating temperature, 60° C; feed and eluant (distilled water) flow rate of 0.25 mL/cm^2/min.

Fractions of 1.32 mL each, starting from tube 22 and up to tube 55 were collected for each sample loading. Tubes 22 to 32 were combined together to form fraction A, and tubes 33 to 55 to form fraction B (Figure 17).

Initially, the individual 1.32 mL fractions were analysed for sugar content to plot the separation chromatogram (Figure 17). Once this had been established, only the combined fractions, A and B, were collected and analysed for

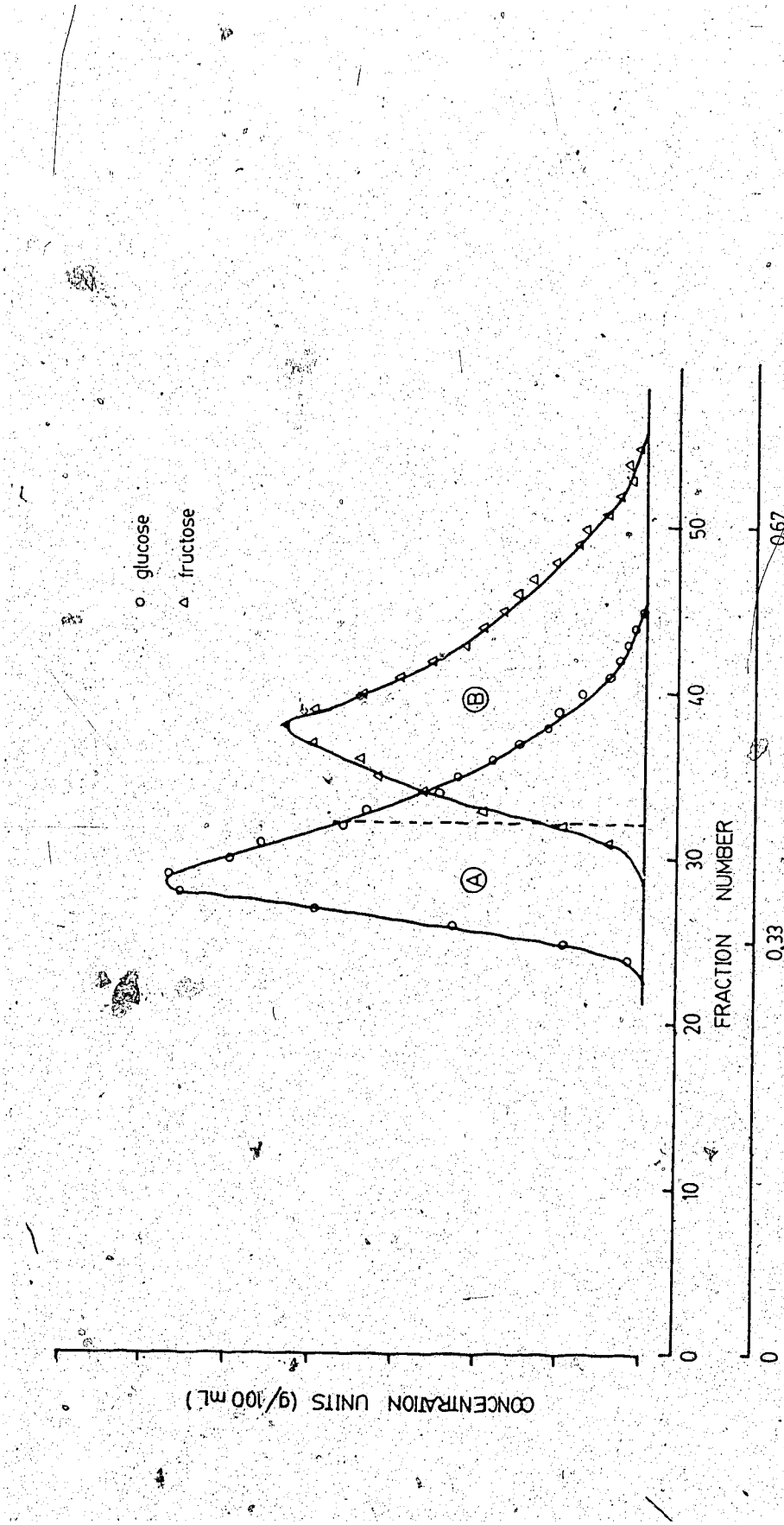


Figure 17. Separation of glucose from fructose with calcium-charged resin. Ⓐ Glucose fraction; Ⓑ Fructose fraction.

pH, colour and sugar content.

4.2.3 Isomerisation

4.2.3.1 Materials

Dextrose crystals ($C_6H_{12}O_6$) anhydrous, ACS grade, were obtained from Fisher Scientific Co., Fair Lawn, NJ.

Analytical grade anion exchange resin, AG 1 - X4, in OH^- form (See Appendix I for conversion process and Appendix II for specifications) were obtained from Bio-Rad Laboratories (Canada) Ltd., Mississauga, O[illegible]

4.2.3.2 Equipment

Pharmacia jacketed chromatography column K 26/40 complete with flow adapters, reservoirs (R 15 and R 25) and 3-way valves, Pharmacia Canada Ltd., Dorval, P.Q.

Braun Thermomix 1441 circulator, B. Braun, Melsungen, West Germany.

4.2.3.3 Procedure

Approximately 100 mL of an anionic exchange resin in the OH^- form was packed into the chromatography column and sweetened-on with dextrose solution according to the 'resin packing procedure' outlined in Appendix I. A Mariotte flask set-up with ascending chromatography was used to maintain the flow rate.

Fraction A from the preceeding separation process

was isomerised at 55° C and a flow rate of 0.10 mL/cm^2/min. The isomerised fraction was then analysed for pH and sugar content.

4.2.4 High fructose solution

Product from the isomerisation step was combined with fraction B from the separation step to form the final high fructose solution. The combined solution was analysed for pH, colour and sugar content.

4.3 Beet colourant studies

4.3.1 Fractionation of beet colourants

4.3.1.1 Fractionation with Sephadex G - 25

4.3.1.1.1 Materials

100 mL burette

Sephadex G - 25 dextran beads (See Appendix II for specifications), Pharmacia Canada Ltd., Dorval, P. Q.

4.3.1.1.2 Procedure

The following procedure was based on the method of Vamos and Pozsar (1968). Rehydrated Sephadex G - 25 dextran beads were packed into a burette. A sample of diluted beet molasses was carefully applied onto the top of the column and elution with distilled water was carried out at a flow rate of 1.0 mL/min.

The colour fractions were collected and scanned with a Beckman spectrophotometer at different wavelengths.

4.3.1.2 Fractionation with Amberlite XAD - 2 resin

4.3.1.2.1 Materials

100 mL burette

Amberlite XAD - 2 resin (See Appendix II for specifications), Terochem Laboratories Ltd., Edmonton, Alta.

95% methanol and 95% methanol containing 0.01 N HCl.

4.3.1.2.2 Procedure

The following procedure was based on the method of Cookson *et al.* (1970). Amberlite XAD - 2 resin, equilibrated in 95% methanol, was packed into a burette. The packed column was then washed with approximately 3 bed volumes of 95% methanol before a sample of diluted beet molasses was carefully applied onto the top of the column.

Initially, elution was performed with 95% methanol at a flow rate of 1.0 mL/min until all the unadsorbed colour fraction was eluted. Subsequent elution of the adsorbed colour fraction was performed with 95% methanol containing 0.01 N HCl at similar flow rates.

The colour fractions were collected and scanned with a Beckman spectrophotometer at different wavelength.

4.3.2 Preparation of 'synthetic' colourants

4.3.2.1 Materials

Dextrose crystals ($C_6H_{12}O_6$) and sodium hydroxide

pellets, ACS grade, were obtained from Fisher Scientific Co., Fair Lawn, NJ.

D - lysine and glycine were obtained from Sigma Chemical Co., St. Louis, MO.

4.3.2.2 Procedure

The 'synthetic' colourants were prepared according to the method described by Cookson *et al.* (1970).

4.3.3 Wavelength scan of 'synthetic' and beet molasses colourants

4.3.3.1 Materials

Beckman DU - 8 spectrophotometer, Beckman Instruments Inc., Fullerton, CA.

Millipore Swinnex - 13 filter holder and 0.45 µm type HA membrane filters, Millipore Ltd., Mississauga,

4.3.3.2 Procedure

Operating conditions

The spectrophotometer was operated in the program mode under the following conditions:

- reading in Absorbance units
- slit width of 0.50 nm
- wavelength scan speed of 50 nm
- starting wavelength of 700 nm
- final wavelength of 240 nm

Wavelength scan

The colourants were filtered through a 0.45 µm membrane filter (Millipore) before wavelength scans were performed. Appropriate dilution of samples was made when necessary so that the absorbance readings were kept below 4.0. The instrument was blanked against appropriate solutions of eluants used in the colour fractionation of beet molasses and against water in the case of 'synthetic' colourants.

4.4 Analytical technique

4.4.1 High performance liquid chromatography (HPLC) analysis of sugars

4.4.1.1 Reagents

Acetonitrile and methanol, HPLC grade, Fisher Scientific Co., Fair Lawn, NJ.

Fructose, dextrose and sucrose, ACS grade, Fisher Scientific Co., Fair Lawn, NJ.

Raffinose and mannose, Aldrich Chemical Co., Milwaukee, WI.

Millipore Milli-Q water system, Millipore Corp., MA.

4.4.1.2 Equipment

HPLC unit

1. Beckman Model 110 A pump, Beckman Inc., Fullerton, CA.

2. Whatman Solvecon precolumn, 25 cm X 4.6 mm ID packed with 37 - 53 µm silica gel and Whatman Guard column, 7 cm X 4.6 mm ID packed with 30 - 38 µm octadecylsilane coated pellicular beads, Whatman Inc., Clifton, NJ.

3. Waters µBondapak Carbohydrate column, 30 cm X 3.9 mm ID packed with 10µm packing, Waters Scientific Ltd., Dorval, P.Q.

4. Pharmacia Refractive Index Model 1103 P monitor, Pharmacia Canada Ltd., Dorval, P.Q.

5. Polyscience Polytemp Model 90 circulator, Polyscience Corp., Niles, IL.

6. Cole Parmer Model 355 recorder, Cole Parmer, Irvine, CA.

Sample preparation equipment

1. Millipore Swinnex - 13 filter holder and 0.45 µm type HA membrane filters, Millipore Ltd., Mississauga, ONT.

2. Waters Sep-Pak C_{18} cartridges, Waters Scientific Ltd., Dorval, P.Q.

4.4.1.3 Procedure

4.4.1.3.1 Chromatographic equipment

The HPLC system used in this research was an isocratic set-up consisting of a Beckman Model 110 A pump and equipped with a Pharmacia Model 1103 P refractive index monitor detector. The HPLC was fitted with a Whatman Solvecon column before the injector. A Whatman Co:Pell ODS guard column was fitted prior to the Waters µBondapak Carbohydrate column for protection.

A Polytemp circulator bath was used to maintain the temperature of the detector at 25° C.

Operating conditions

The following conditions were employed in the analysis of sugars by HPLC:

- the mobile phase composition of acetonitrile:water (Millipore Milli-Q system) was 75:25 ratio (w/w),

- flow rate of the mobile phase was 1.80 mL/min

4.4.1.3.2 Sample preparation

Samples were first diluted to a 1 to 5% range of sugar concentration (a pocket refractometer was used as an approximate check). The diluted samples were filtered through a 0.45 µm membrane filter (Millipore) and then through a Waters Sep-Pak C_{18} cartridge according to Waters Sep-Pak cartridges instruction sheet No. IP82904.

The prepared samples were then ready for the analysis.

4.4.1.3.3 Sample analysis

A minimum of 5 replicates for each sample (20 µ[redacted] were analysed for sugar content according to the method described by Damon and Pettitt (1980).

4.4.1.3.4 Identification and quantitation of chromatograms

Identification of sample components were carried out by comparing their retention times with those of pure standards. These were further confirmed by using a technique called 'spiking' which involved addition of a known standard to a sample and observing which of the peaks on the chromatogram increased in height.

Quantitation of sugars in the sample was performed using peak areas and calibration curves of standard sugar solutions. All calibration curves were linear in the 1 to 5% (w/v) range. Figure 18 and 19 show a typical chromatogram of

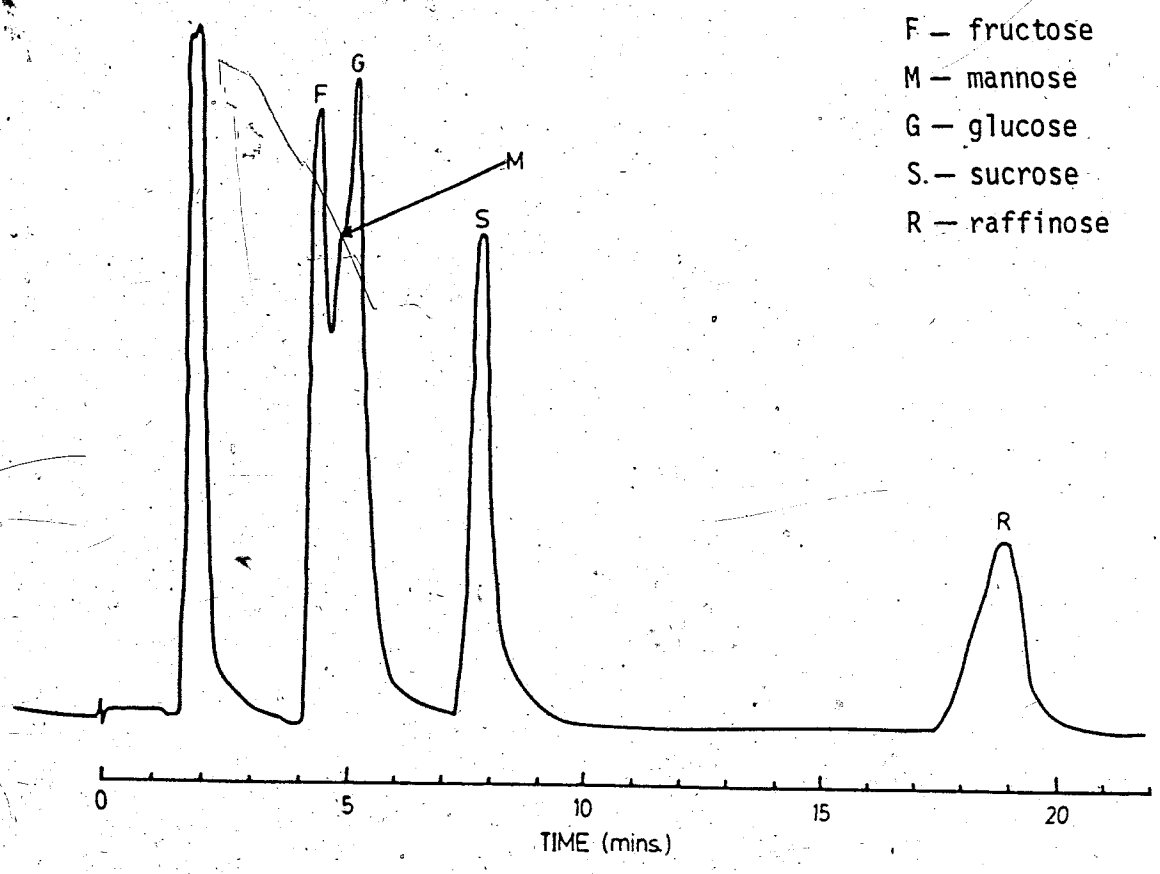


Figure 18. A typical HPLC chromatogram of a mixture of fructose, mannose, glucose, sucrose, and raffinose.

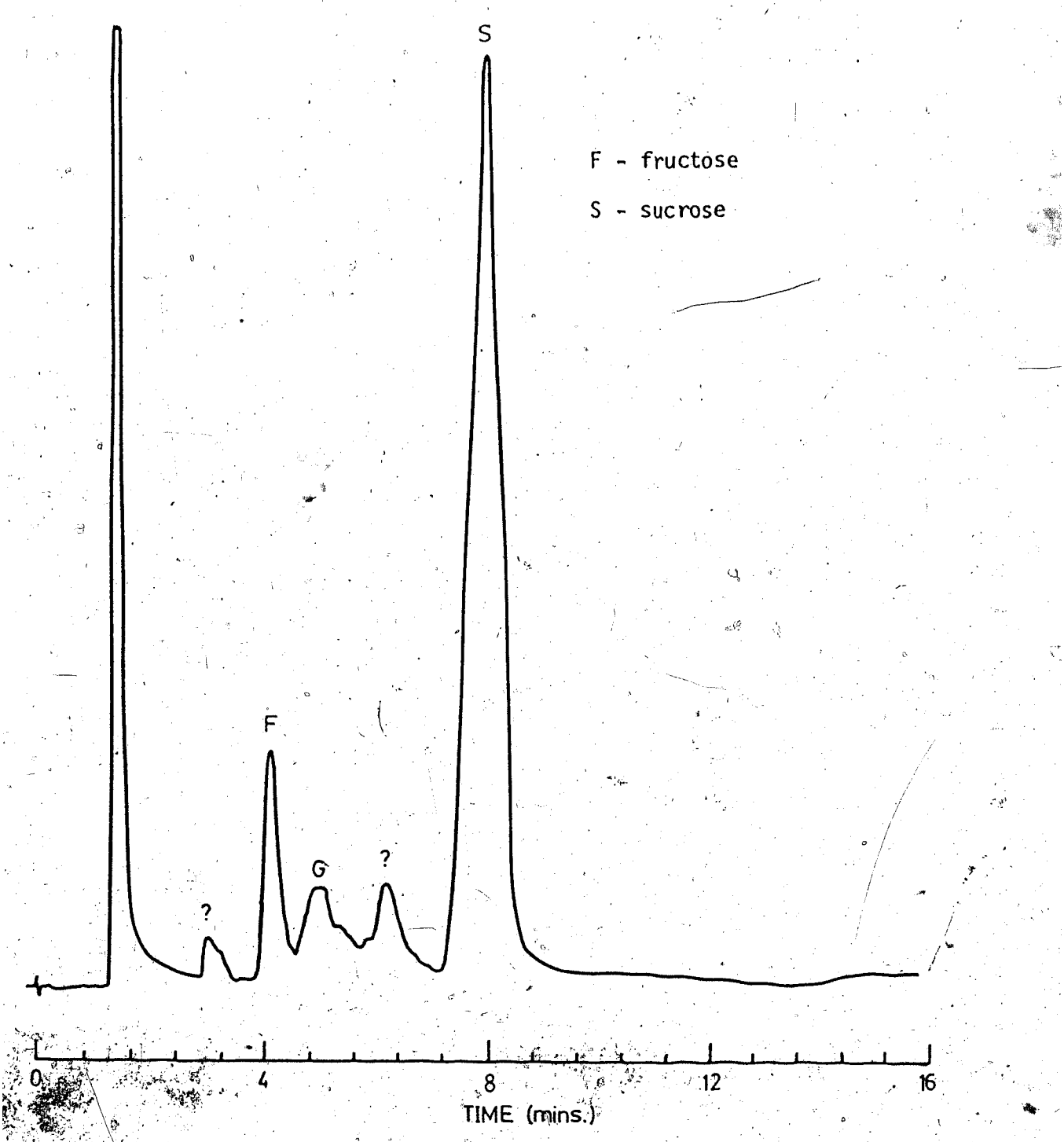


Figure 19. A typical HPLC chromatogram of diluted beet molasses.

a mixture of fructose, mannose, glucose, sucrose and raffinose, and of diluted beet molasses, respectively.

4.4.2 Spectrophotometric measurements

4.4.2.1 Reagents

4 N HCl and NaOH solution

Millipore Milli-Q water system, Millipore Corp., MA.

4.4.2.2 Equipment

Spectronic 20 spectrophotometer, Bausch and Lomb, New York, NY.

Millipore Swinnex - 13 filter holder and 0.45 µm type HA membrane filters, Millipore Ltd., Mississauga, Ont.

4.4.2.3 Procedure

4.4.2.3.1 Sample preparation

Samples were adjusted to a pH of 7.0 and filtered through a 0.45 µm membrane filter (Millipore).

4.4.2.3.2 Spectrophotometric colour measurements of samples

Samples were measured at 420 nm according to procedure outlined by the International Commission for the Uniform Methods of Sugar Analysis (ICUMSA) (Schneider, 1979).

Appropriate dilution of samples were made when necessary so that the Absorbance values were kept within the range of 0.05 to 1.00. Three replicates were measured for each sample.

4.4.3 Ash content measurements

Ash content was measured in conductivity units (micromho/cm) at 25° C with a Wescan Model 212 conductivity meter equipped with a model 219 - 700 dip cell.

Standard solutions of NaCl were made to obtain calibration curves (Figures 20 and 21) and readings of samples were then expressed in terms of NaCl concentrations.

The dip-cell and the instrument was calibrated in a KCl standard solution, supplied by the manufacturer, according to the instrument's operation manual.

4.4.4 pH measurements

pH measurements of samples were made with an expanded scale Fisher pH meter model 230.

4.4.5 Total solids measurements

Total solids (t.s.) contents of samples (5 replicates) were determined by drying samples in aluminium dishes for 24 hours at 70° C and 17.4 kPa (25" Hg) vacuum.

T.s. was calculated as a percentage of the original sample weight.

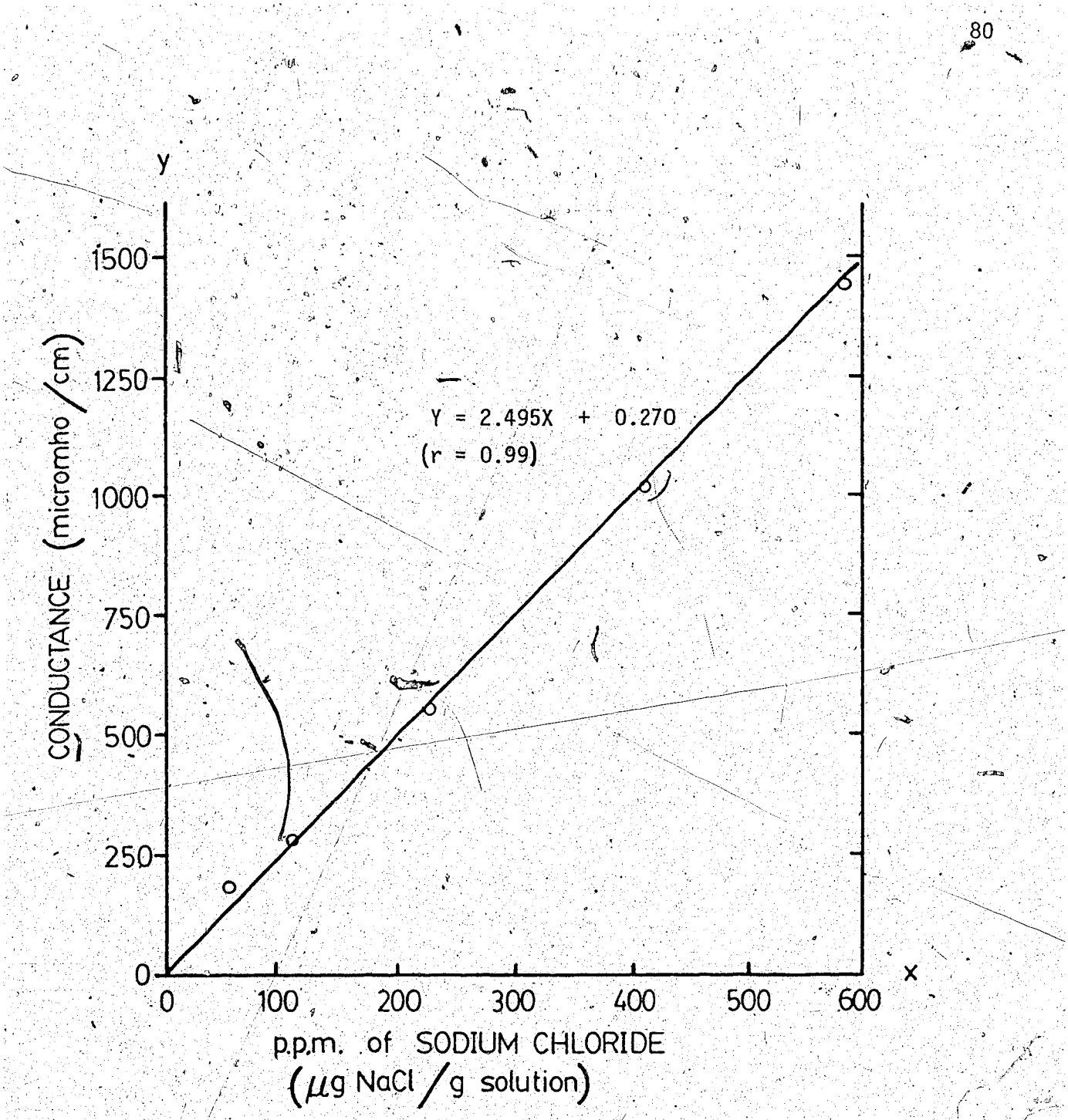


Figure 20. Calibration curve for 0 to 600 p.p.m. of NaCl solution

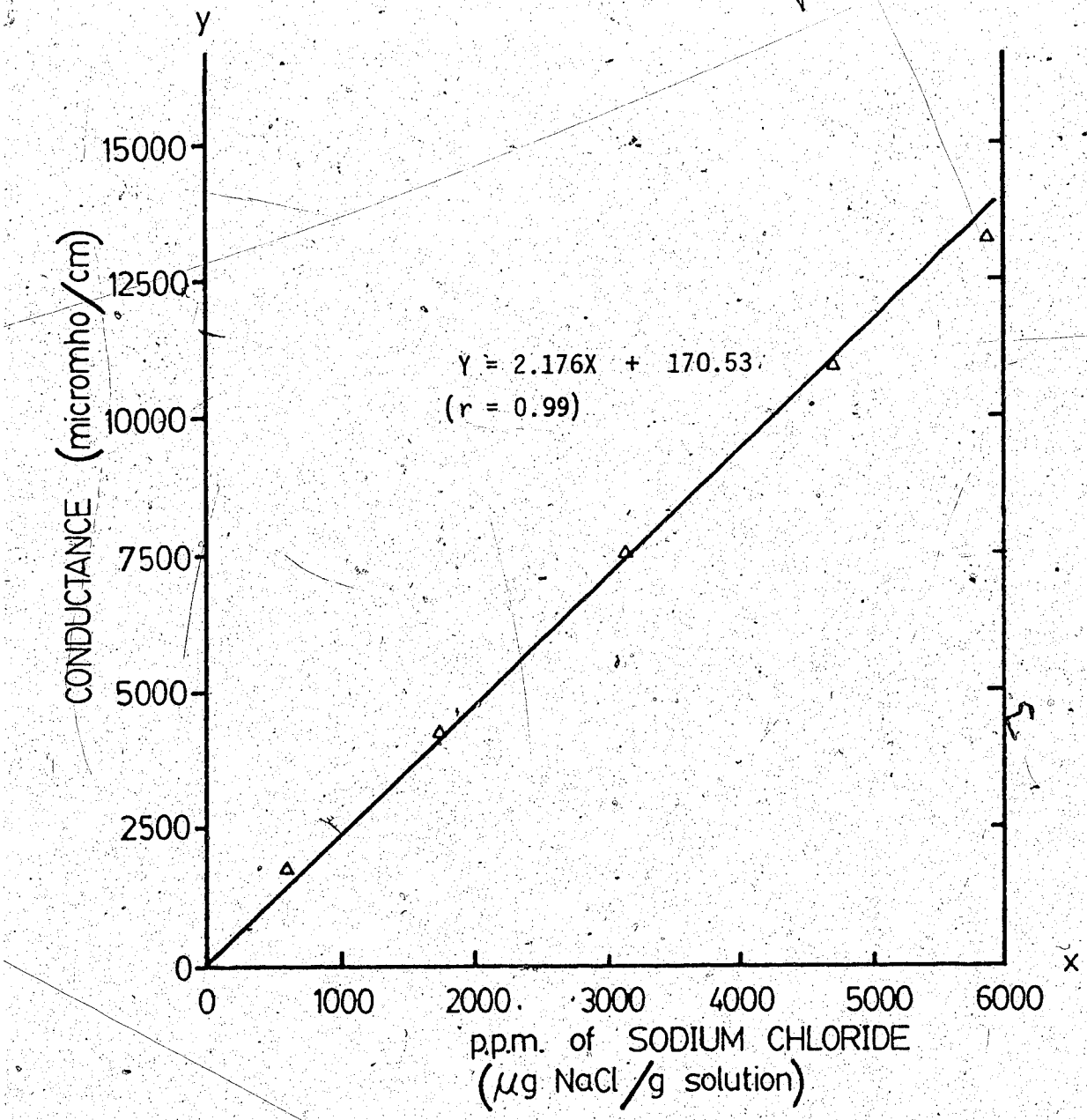


Figure 21. Calibration curve of 0 to 6000 p.p.m. of NaCl solution.

5. RESULTS AND DISCUSSION

5.1 Composition of Alberta beet molasses

The composition of Alberta beet molasses together with a typical composition of beet and cane molasses are shown in Table 1. Sucrose content of Alberta beet molasses was not significantly different from that of the typical beet molasses. The amount of sucrose present in the final molasses is a reflection of the degree of exhaustion of the molasses during the sugar boiling and crystallisation processes, especially the final sugar crystallisation process in the low raw crystalliser. The degree of exhaustion ~~of molasses~~ varies but is mostly governed by the retail price of sugar and what use is made of the ~~final product~~, molasses.

Neither raffinose nor glucose was found in Alberta beet molasses. However, its fructose content was significantly higher than the amount of invert sugar of beet molasses reported by McGinnis (1951) but was comparable to that of cane molasses reported by Meade and Chen (1977). The presence of invert sugars in molasses can be attributed to the storage of raw beets and/or processing conditions employed in the sugar manufacturing process. Studies have shown that repeated freezing and thawing during storage could cause an increase in invert sugars in the beet (Ivory, 1980). The higher fructose content could be an asset in the production of HFS from

molasses as fructose is the desired monosaccharide in the final product. However, the quantity of fructose in the HFS was also dependent on the processing conditions used in its manufacture such as the acidic condition during the hydrolysis or basic condition during the isomerisation.

Ash content of Alberta beet molasses as measured by the conductivity method was significantly lower than that of the California beet molasses reported by McGinnis (1951). This difference could be due to either the variety of the beets, cultural practices, and the location they were grown, or the technique used for ash analysis. It was not clear what method was employed to determine ash content of California molasses, but it is possible that other techniques such as wet ashing with sulphuric acid might give a higher value for ash content. It was interesting to note that a sediment was present in diluted Alberta beet molasses. This sediment could be due to the presence of lime salts as liming was practised during the sugar manufacturing process. Because of low solubility of lime salts their presence might not be detected in the conductivity measurements and hence low ash content of the molasses was recorded.

5.2 Ultrafiltration

Ultrafiltration was used in the purification process to remove beet molasses colourants and suspended solids. The process removed 65.7% of the original colour of the diluted

raw beet molasses (Figure 22). The permeate appeared much lighter in colour and required much less quantities of activated carbon for further colour removal than the original diluted molasses. Since the ultrafiltration membranes used were of 10,000 nominal M. W. cut-off, it was quite evident that about 65% of the colourants present in Alberta beet molasses had M. W. greater than 10,000. However, with continued use the selectivity of the membranes might change, allowing the retention of molecules of smaller M. W. at the expense of filtration efficiency.

In addition to colour removal, sediment of lime salts in the diluted molasses was also removed. This was evident by the clarity of the permeate, which was medium brown in colour (Figure 23), and the appearance of the sediment on the ultrafiltration membranes and in the retentate.

Ultrafiltration also resulted in the reduction of 10.9 and 26.4% of fructose and sucrose, respectively, in the permeate (Figure 24, C). A greater reduction of sucrose in the ultrafiltrate could be due to the larger size of its molecule as compared to fructose, or to possible complex formation between sucrose and beet impurities e.g. beet colourants, colloidal compounds, etc., reducing its ability to traverse the membrane.

There was no change in pH of the molasses after ultrafiltration (Figure 25). This might be construed that colourants and other impurities removed in the process were

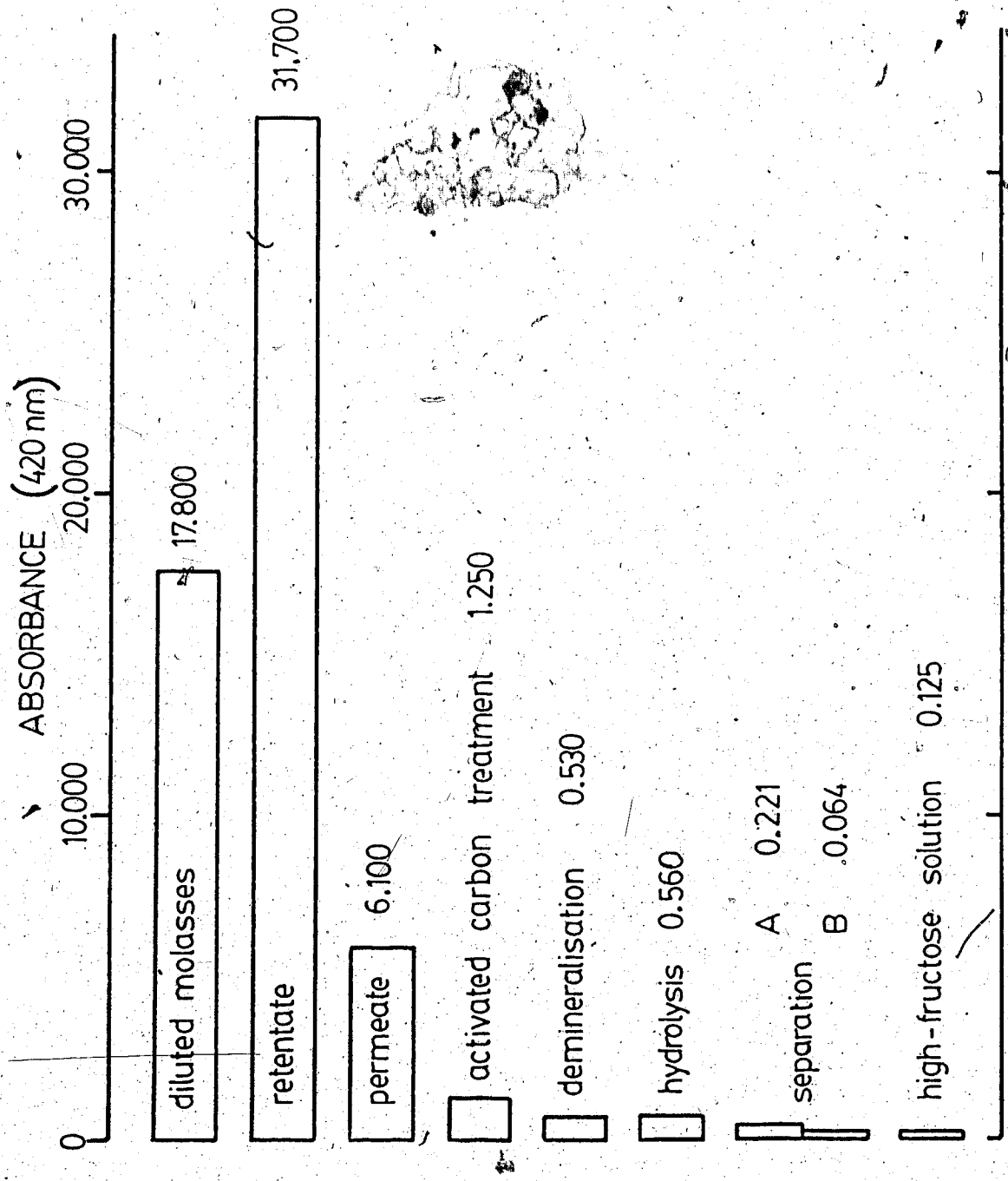


Figure 22. Colour profile of diluted molasses and subsequent products following each unit operation in the production of HFS. (A = glucose fraction; B = fructose fraction.)

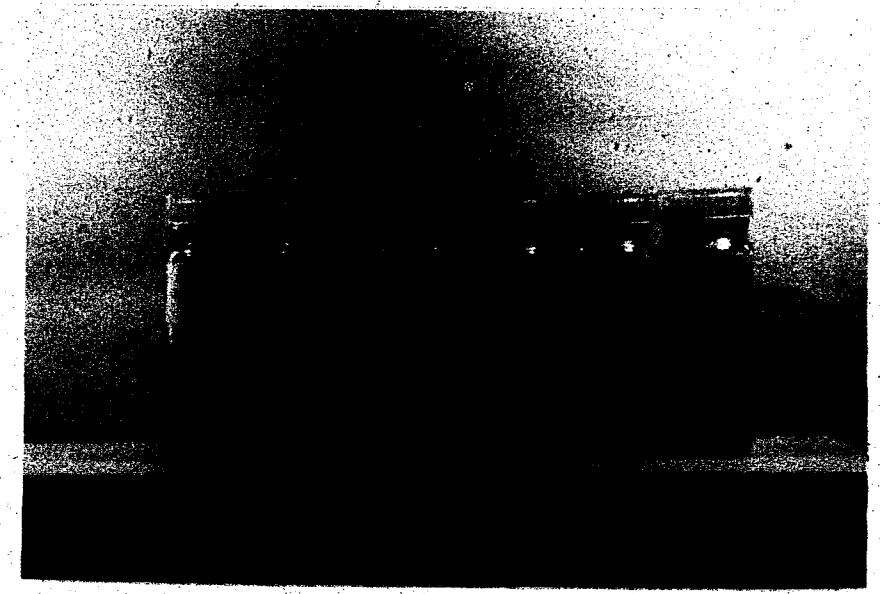


Figure 23. Colour profile of molasses after some unit operation in the HFS process. From left to right: Diluted molasses, retentate, permeate, activated carbon treated product, demineralisation product and hydrolysed product.

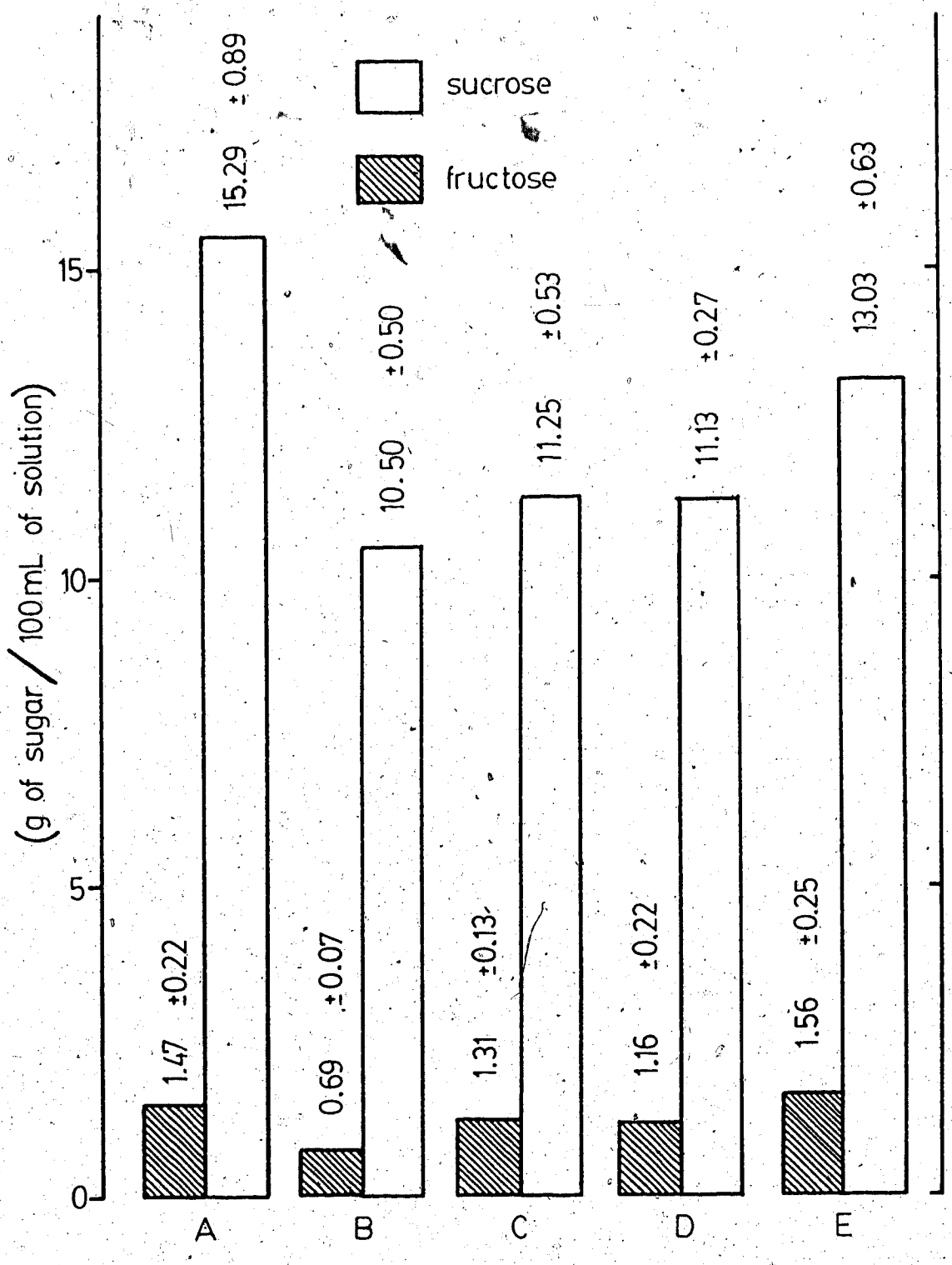


Figure 24. Sugar profile of the product after each unit operation in purification process. A - diluted molasses, B - retentate, C - permeate, D - permeate after activated carbon treatment, E - permeate after activated carbon and demineralisation treatments.

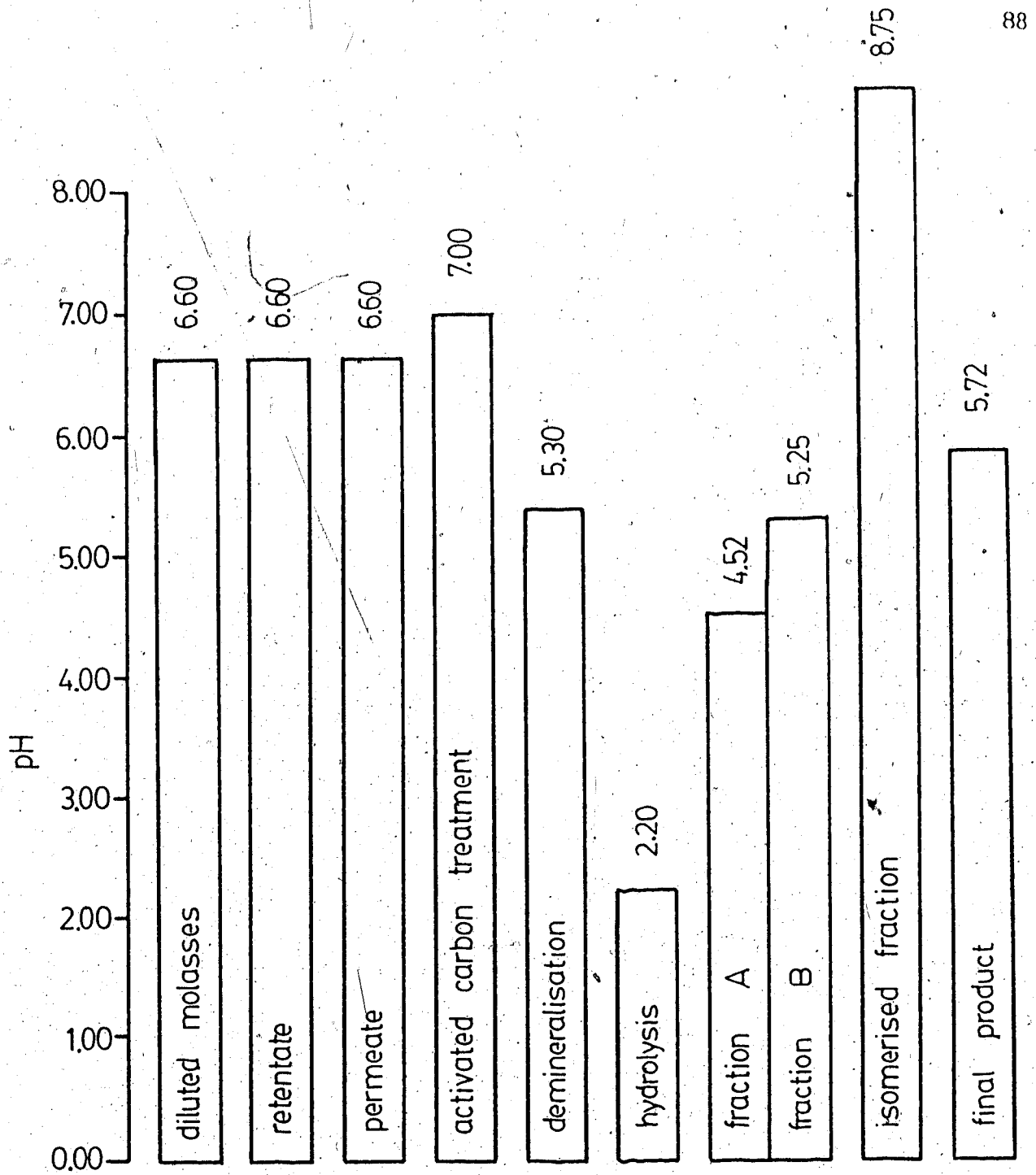


Figure 25. pH profile of diluted molasses and the product following each unit operation in the production of HFS.

basically neutral and played no part in buffering capacity of the molasses. There was a slight increase in the conductivity (ash content) of the permeate after ultrafiltration (Figure 26). Much of the metal ions having small M. W. must have passed through the membranes, resulting in the increase.

Total solids content of the retentate and permeate increased practically in direct proportion with the operating time of the ultrafiltration operation (Figure 27). The increases could be attributed to the changing composition of the feed. Since a single-stage batch-recycle system was used with the retentate being recycled back to the feed tank, as more permeate consisting of water, sugars, inorganic salts, etc. was being withdrawn, the feed composition changed continuously, becoming more concentrated. Increased concentration would reduce the diffusion coefficient in the fluid and increase its viscosity. These reduced the rate of transfer of water to the membrane and hence reduced the flux through the membrane (Cooper, 1980). This was reflected in the increases in the absorbance values of both the retentate and the permeate (Figure 28) and significant decrease in the permeate flux rate (Figure 29). Referring to the equation in section 3.2.1, i.e. $J = K_p (\Delta P - \Delta \Pi)/(R_m + R_b)$, resistance, R_b, due to concentration polarisation and fouling, occurred to a high degree shortly after the commencement of the operation. This was clearly shown by the rapid decline in the permeate flow rate. The phenomenon was further confirmed after

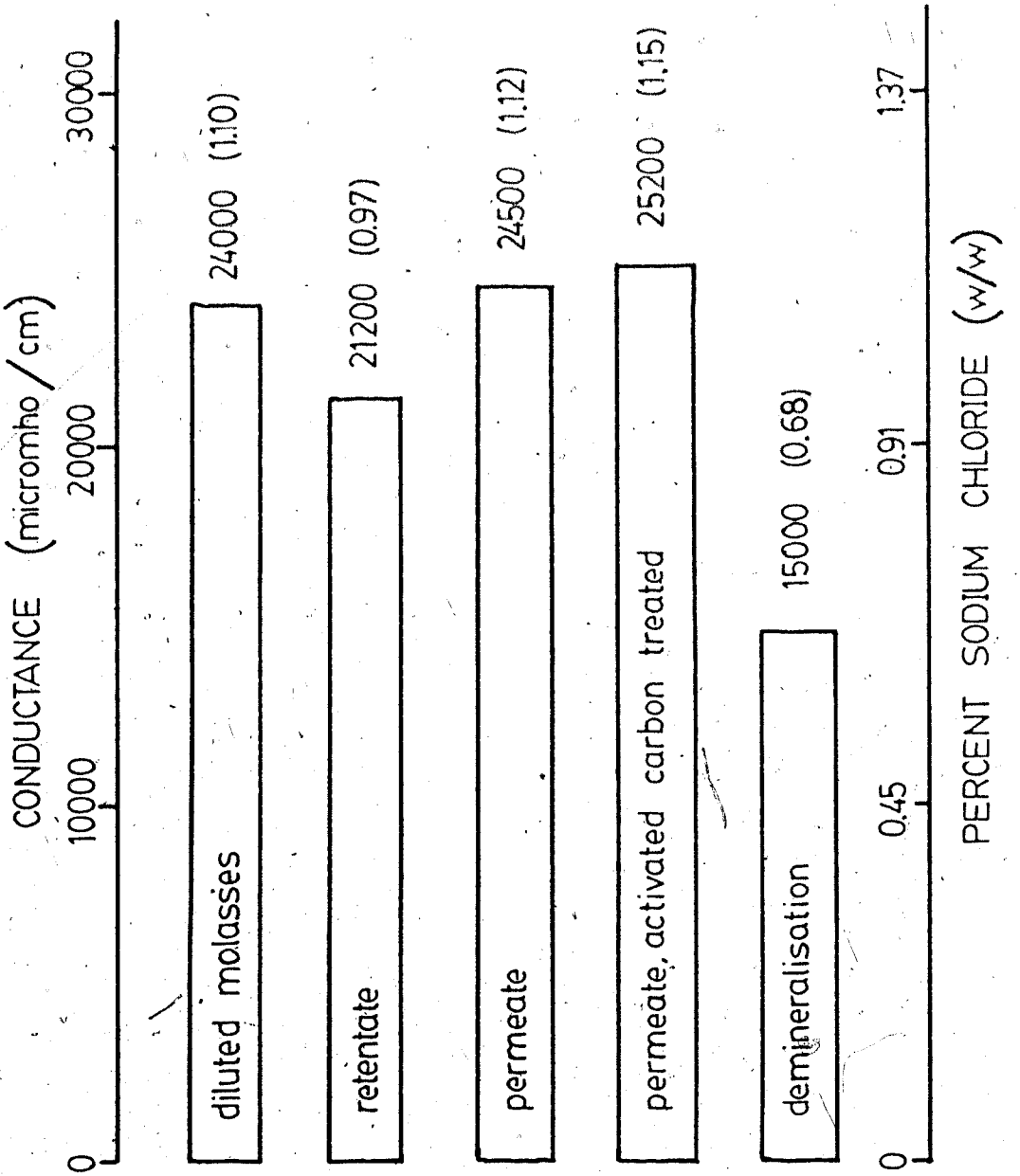


Figure 26. . Ash content (as % NaCl) profile of diluted molasses and the product following each unit operation in the purification process.

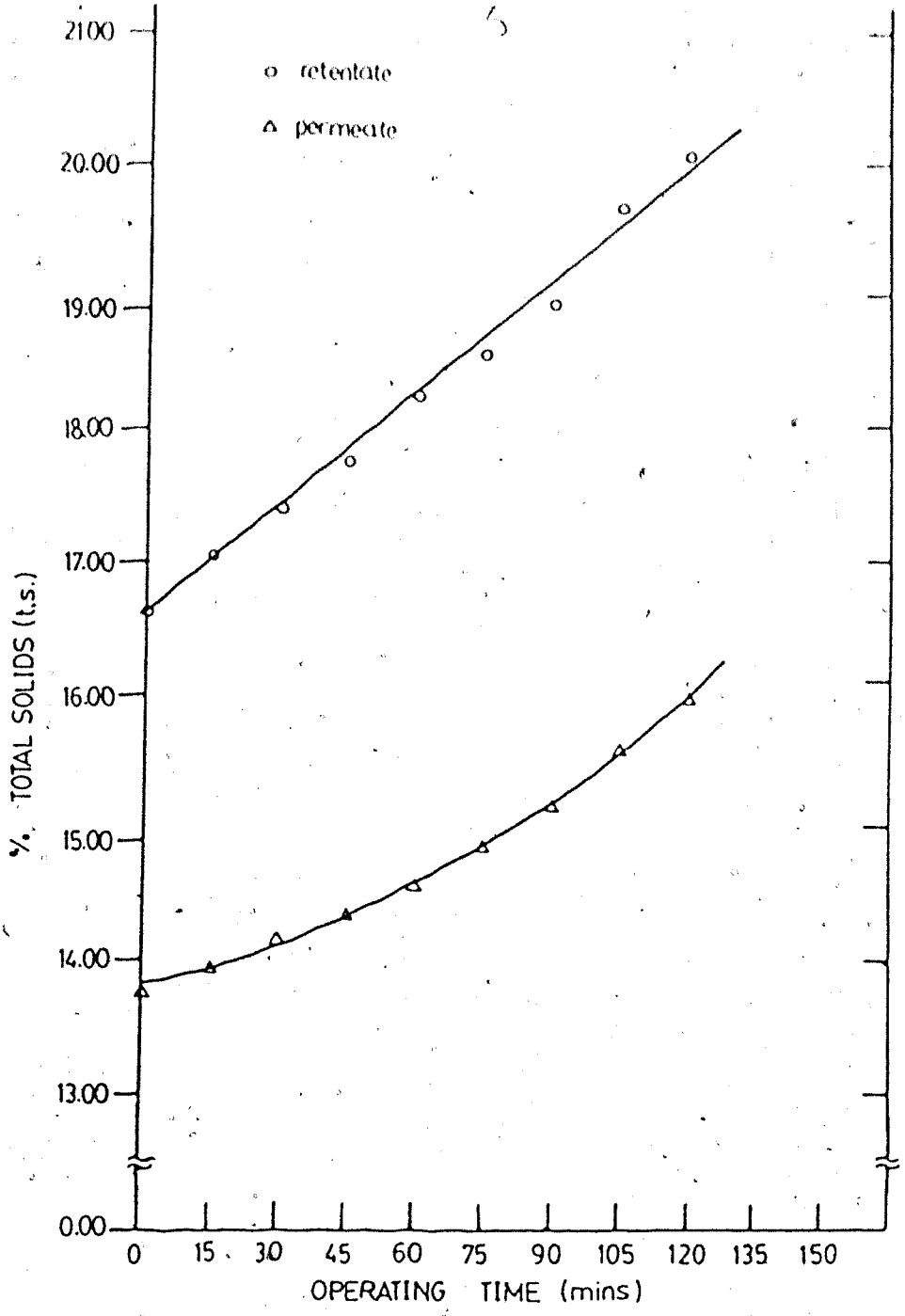


Figure 27. Effect of operating time on total solids content of retentate and permeate of ultrafiltration.

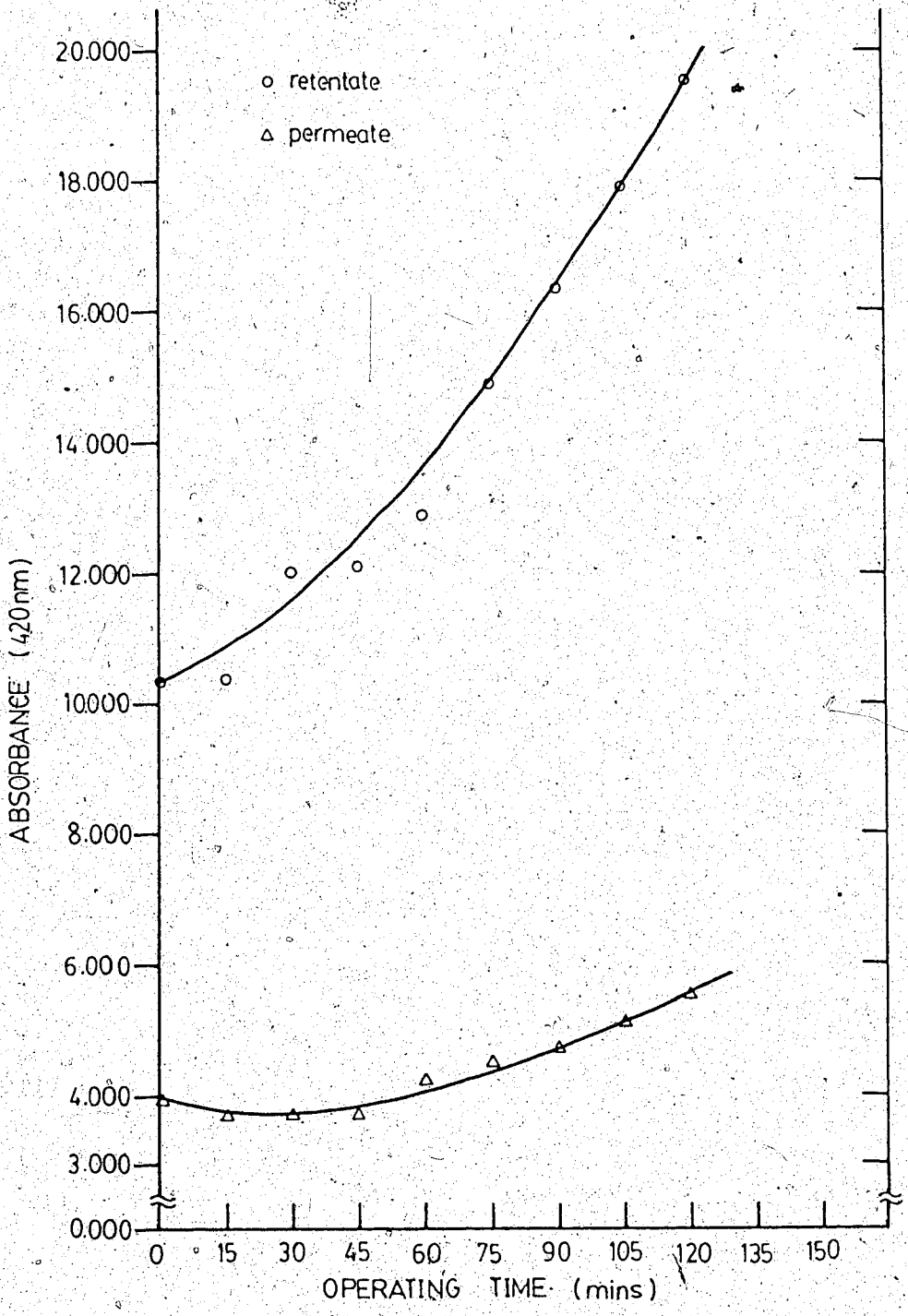


Figure 28. Effect of operating time on colour intensity (absorbance at 420 nm) of permeate and retentate of ultrafiltration.

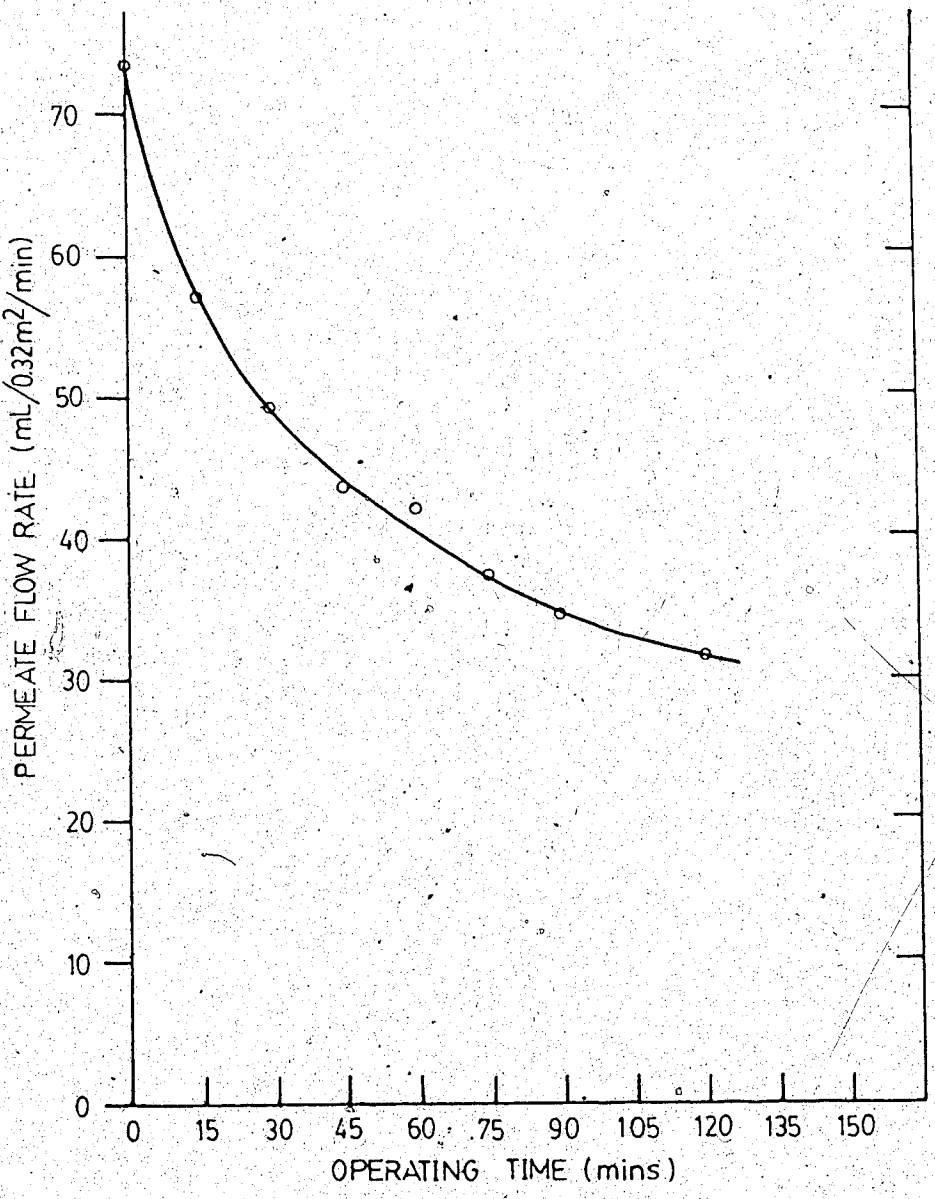


Figure 29. Effect of operating time on the flow rate of permeate (permeate flux) of ultrafiltration operation.

the termination of the operation when the module was decompressed, and the membranes were carefully examined and found to have a significant amount of sediment and a gel-like layer on the surfaces of all the membranes. More substantial accumulation of the materials was found on the membranes closest to the inlet terminal of the module. Another interesting observation was the slight increase in the inlet pressure as the operation progressed. This could also be due to the fouling of the membranes. Cooper (1980) stated that osmotic pressure, $\Delta\Pi$, was a significant factor determining the flux rate in reverse osmosis. This is due to the accumulation of microsolutes, e.g. salts and sugars, which have high osmotic potential. However, in ultrafiltration essentially only macrosolutes, e.g. proteins and colloidal compounds are retained. These macrosolutes have a much lower osmotic potential and hence $\Delta\Pi$ has a negligible effect on the flux rate of the ultrafiltration process.

The loss of sugars during ultrafiltration, amounting to 55% of the original sugar content (Table 3), was the highest in the entire process. This loss occurred through the discarded retentate as the recycling was stopped once the volume concentration ratio of 2.50 had been reached. The loss could probably be minimised on a commercial scale by using either a multi-stage series or parallel operation as shown in section 3.2.2.

Table 3. Sugar loss and gain at various steps of processing calculated based on sugar content of the starting material for each unit operation.

Processing step	Loss %	(Gain %)		
	Overall process	Sucrose	Fructose	Glucose
Purification of molasses				
Ultrafiltration	55.01	55.85	46.26	—
Activated carbon	5.97	4.89	15.19	—
Demineralisation	(18.62)	(16.98)	(34.33)	—
High fructose syrup production				
Hydrolysis	10.46	—	11.51	9.36
Separation	(22.71)	—	(29.37)	(14.66)
Isomerisation	(2.40)	—	—	—

Some problems associated with prolonged use of the membranes were exceedingly slow flux rate of the permeate (a reduction of about 50% of the original) and staining of the membranes. However, it was found that if the membranes were cleaned according to the manufacturer's instructions shortly after use continued operation should not affect their intrinsic flux rate significantly.

5.3 Powdered activated carbon treatment

Powdered activated decolourising carbon was used to remove carbon-adsorbable colourants and other impurities, e.g. colloidal materials, in beet molasses as another purification step before it was further purified by demineralisation. The carbon treatment removed 27.5% of the original colour of the diluted beet molasses (See Figure 22). The treatment also removed 6.0 % of sugars (sucrose and fructose) through adsorption by the powdered carbon (See Figure 24, D and table 3). There was a slight increase of pH, from 6.60 to 7.00, after carbon treatment (See Figure 25). Ash content also increased slightly from 1.12% to 1.15%, calculated as % NaCl (See Figure 26). This is due to the ineffectiveness of the activated vegetable carbon in the removal of ashes as compared to bone char (Mantell, 1968; Heroes, 1974, 1977). Hence, an additional purification step using mono-bed resins to remove the remaining ashes was necessary before the molasses could be hydrolysed. The colourant adsorption

characteristic of powdered carbon could be described by the Freundlich equation (Mantell, 1951): $\frac{x}{m} = KC^{\frac{1}{n}}$. For the adsorption of ultrafiltrated Alberta beet molasses on 'Norit' powdered decolourising carbon (Figure 30) the equation was $y = 33.42x^{0.304}$. The adsorption isotherm plot based on this equation could be dsecribed by the equation $x = -0.294 + 0.860c$ (Figure 31).

It is apparent from the curves shown in Figure 30 that successive carbon dosage in equal increments produced a gradually diminishing effect on the ability of the carbon to remove the beet colourants. The limiting dosage was reached at approximately 35% of carbon (w/w) based on the total solids of the solution to be treated (Figure 30).

The use of synthetic organic resins for the decolourisation of ultrafiltrated molasses was also studied. Several types of resins were tried. These included Rexyn 201, Cl^- form (a strong base anionic exchanger), Amberlite IR - 112, Na^+ form (a strong acid cationic exchanger), Dowex MWA - 1, Cl^- form (a weak base anionic exchanger), and Duolite S - 761 adsorbent resin (a granular crosslinked phenol-formaldehyde resin). Only Rexyn 201 was found effective in the removal of beet colourants and its decolourisation curve is shown in Figure 30. However, on an equal adsorbent loading basis (i.e. equal weight of adsorbent/unit volume of solution to be treated), Rexyn 201 was only about half as effective in the removal of beet colourants as compared to powdered activated carbon. The experiments with synthetic

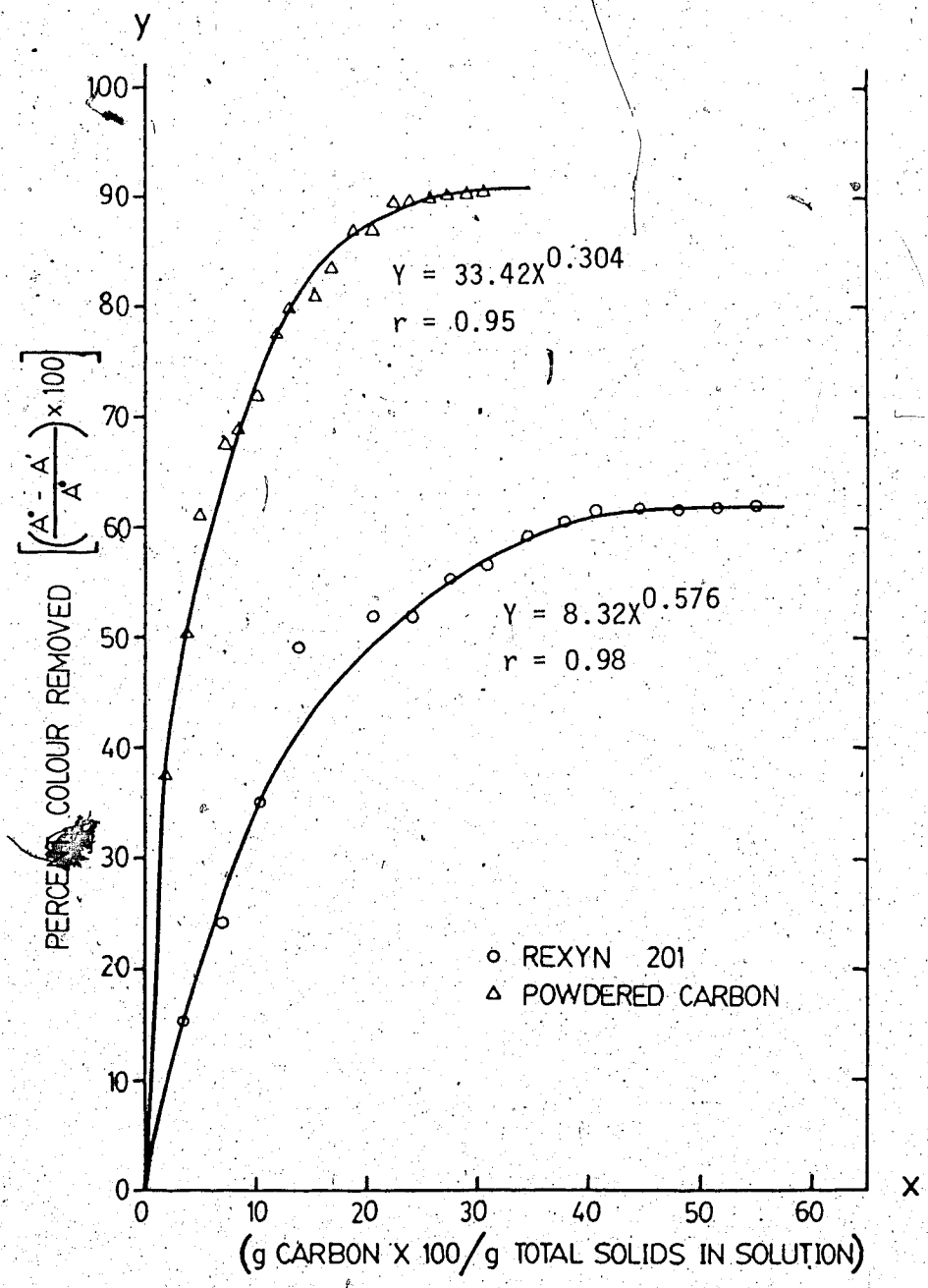


Figure 30. Percent of colour removed from permeate when treated with varying amounts of powdered activated carbon and Rexyn 201 resin.

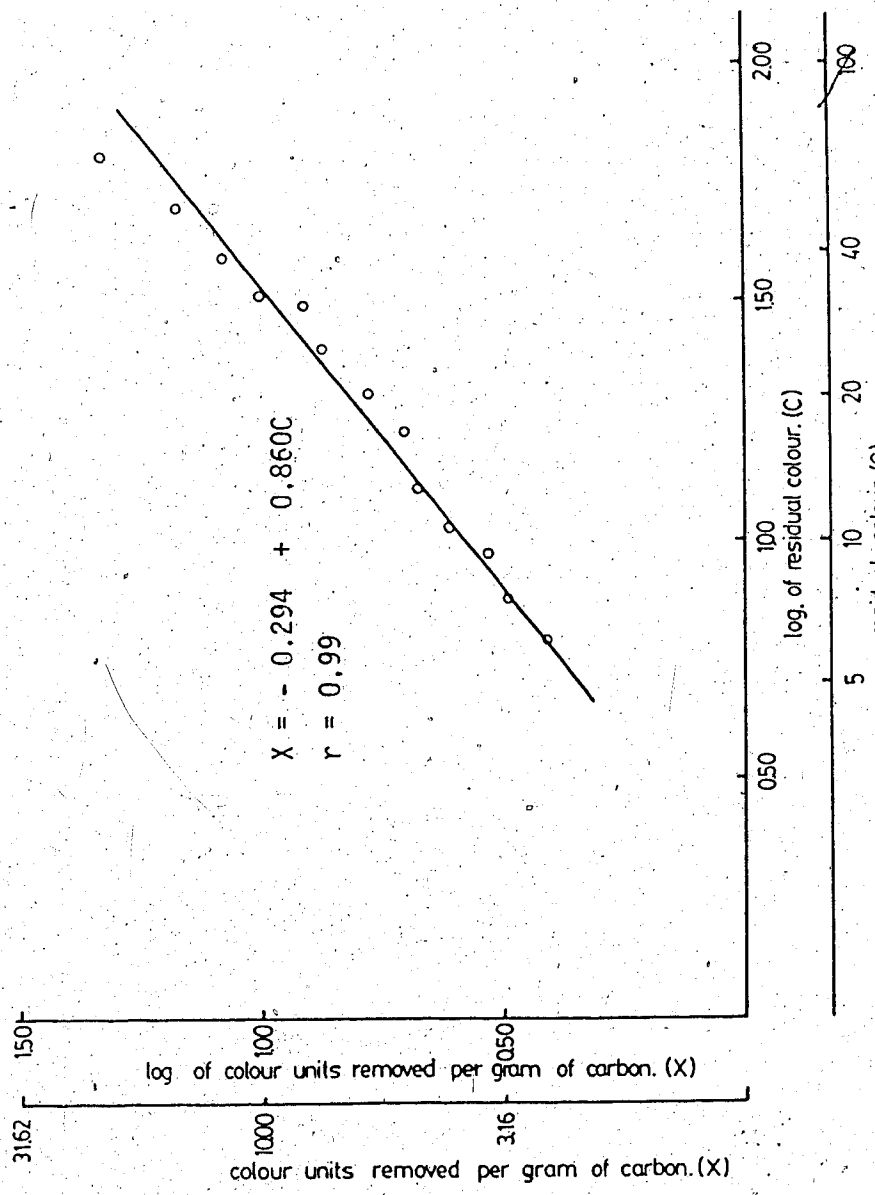


Figure 31. Adsorption (decolourisation) isotherm of ultrafiltrated beet molasses treated with varying amounts of powdered activated carbon.

organic resins also confirmed the results of Tu and Payne (1965) who found that the majority of removable molasses colourants were of a highly anionic nature. This explains the fact that they were more conducive to removal by a strong anionic resin such as Rexyn 201.

Resins have an advantage over powdered activated carbon with respect to their regenerability. However, initial capital costs of the resin might be a deterrent to its wide usage in the sugar industry. The economics of the process will dictate what type of adsorbents to be used.

Colour fractionation studies with either Amberlite XAD - 2 resin or Sephadex G - 25 dextran beads showed that beet molasses colourants were composed of two fractions, a brown and a yellow fraction (Figure 32). Sephadex G - 25 dextran beads have a fractionation range of 100 - 5000 Daltons (See Appendix II for specifications). When diluted raw beet molasses was fractionated on these beads, the brown preceeded the yellow fraction in the elution. This result showed that the brown colourants have much greater M. W.s than the yellow colourants, possibly greater than 5000. The yellow colourants, with M. W.s closer to the 100 - 5000 Daltons range, was eluted much later than the brown colourants.

Fractionation with Amberlite XAD - 2 showed that the yellow colourants were more polar than the brown colourants. This was apparent through the fact that they were more strongly

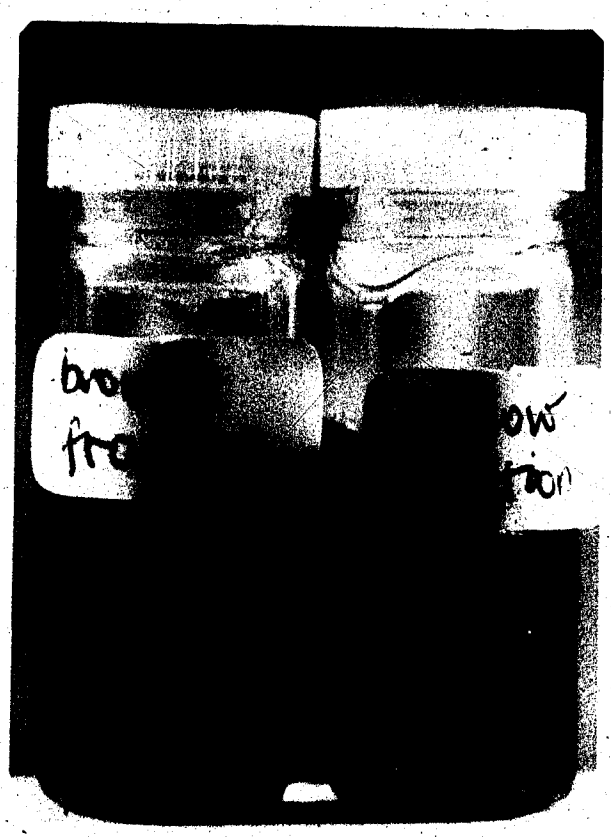


Figure 32. Brown and yellow colour fractions of Alberta beet molasses.

retained on the XAD - 2 resins and had to be eluted with a more polar eluant, ie. 95% methanol containing 0.01 N HCl, whereas elution of the brown colourants required only a 95% methanol.

Ultrafiltration would most likely remove more brown colourants than yellow due to their greater M. W.s. Further, it has been known that activated carbon are more effective in the removal of high M. W. and less polar colourants (Mantell, 1951). Therefore, much of the remaining brown fraction in beet molasses solution would be further removed during the subsequent activated carbon treatment. Nevertheless, some of the yellow colourants could be expected to be removed by activated carbon as well as the adsorption process is not very specific.

In an attempt to elucidate the nature and origin of the beet molasses colourants, spectrophotometric scanning over 700 - 240 nm was performed on the following samples: Diluted beet molasses before and after ultrafiltration, activated carbon treatment and demineralisation, molasses colourants fractionated with Sephadex G - 25 and Amberlite XAD - 2 resins; 'synthetic' colourants prepared by heating glucose, and glucose with lysine or glycine; and, 0.09% (w/v) solution of caramel. The results are shown on Figures 33 to 37.

In all cases, the scans showed strong absorption in the U. V. region. The untreated diluted molasses (Figure 33, curve No. 1) showed peak maxima at 340 and 275 nm. The ultrafiltrate had no detectable peak at 340 nm, but a well defined

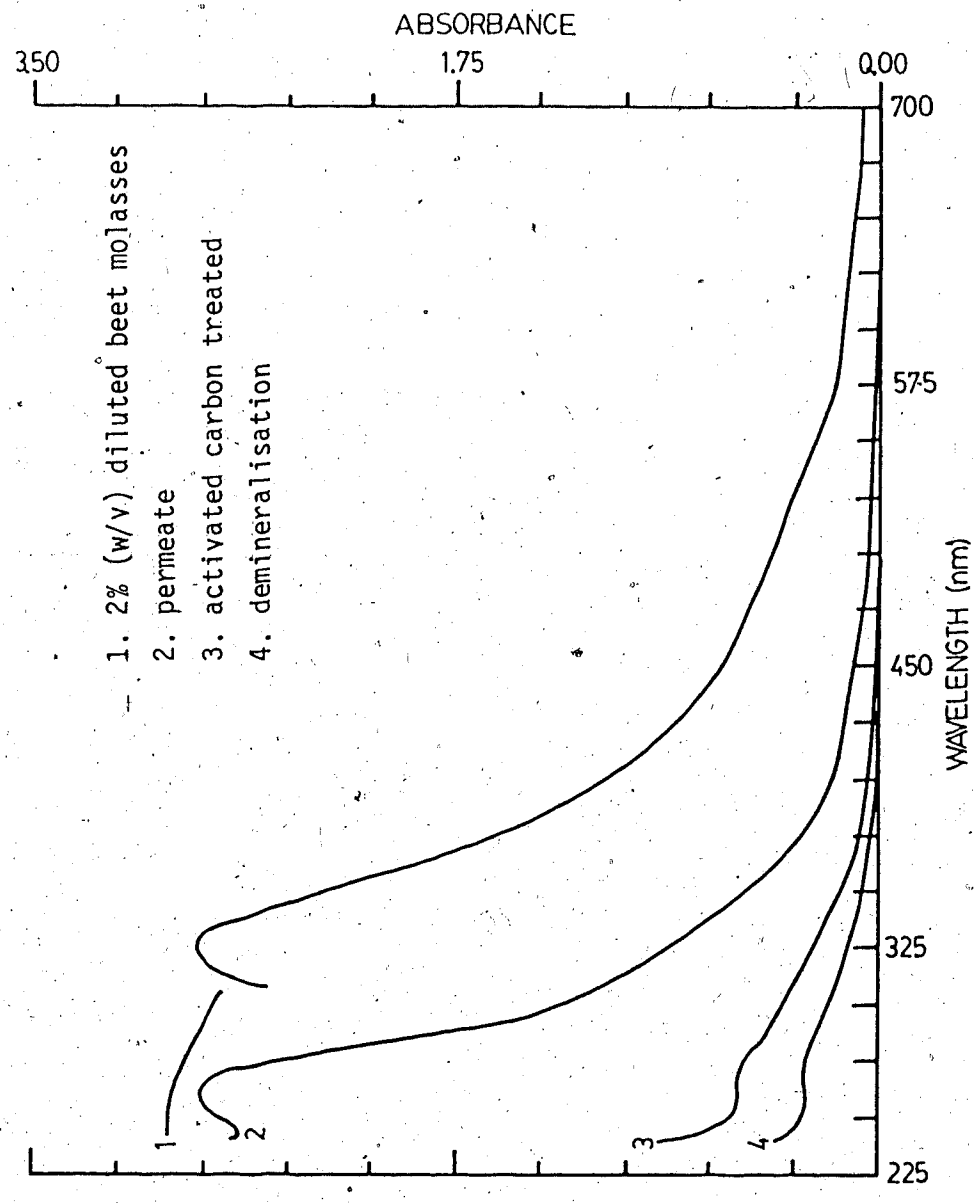


Figure 33. Wavelength scan of Alberta beet molasses before and after ultrafiltration, activated carbon treatment and demineralisation.

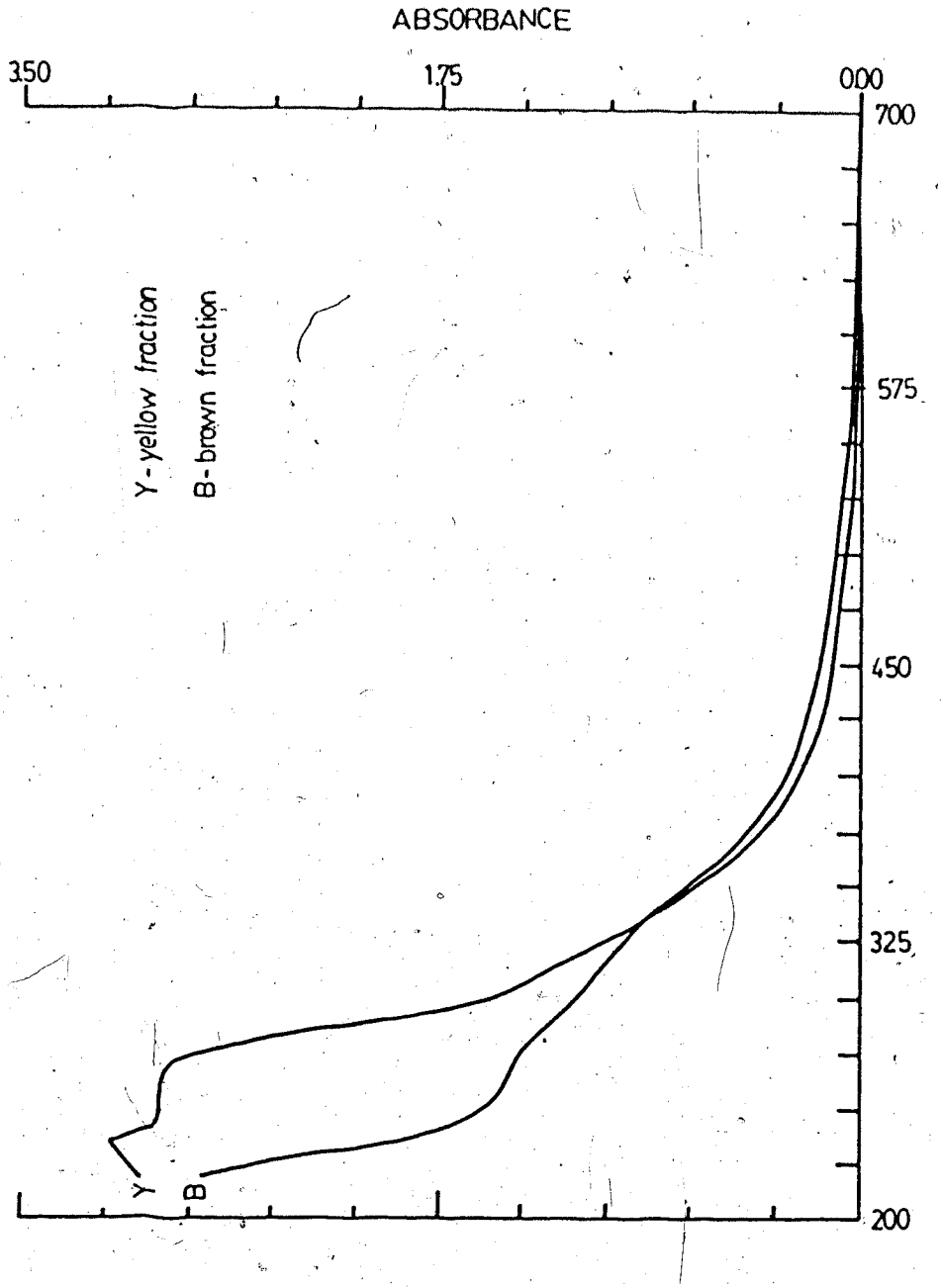


Figure 34. Wavelength scan of yellow and brown colour fractions of beet molasses fractionated on Sephadex G - 25.

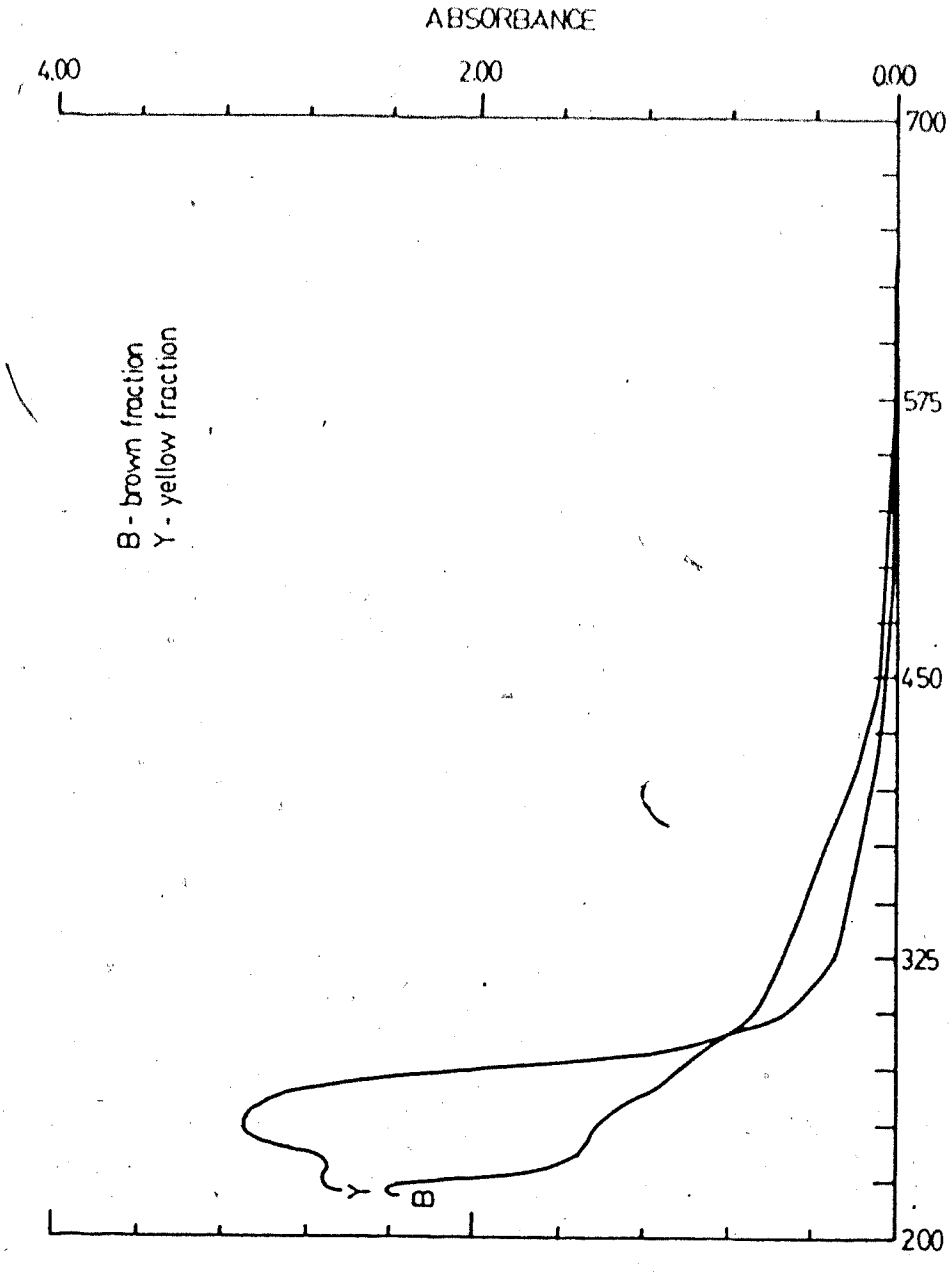


Figure 35. Wavelength scan of the yellow and brown colour fractions of beet molasses fractionated on Amberlite XAD - 2 resin. (Blanked against 95% methanol.)

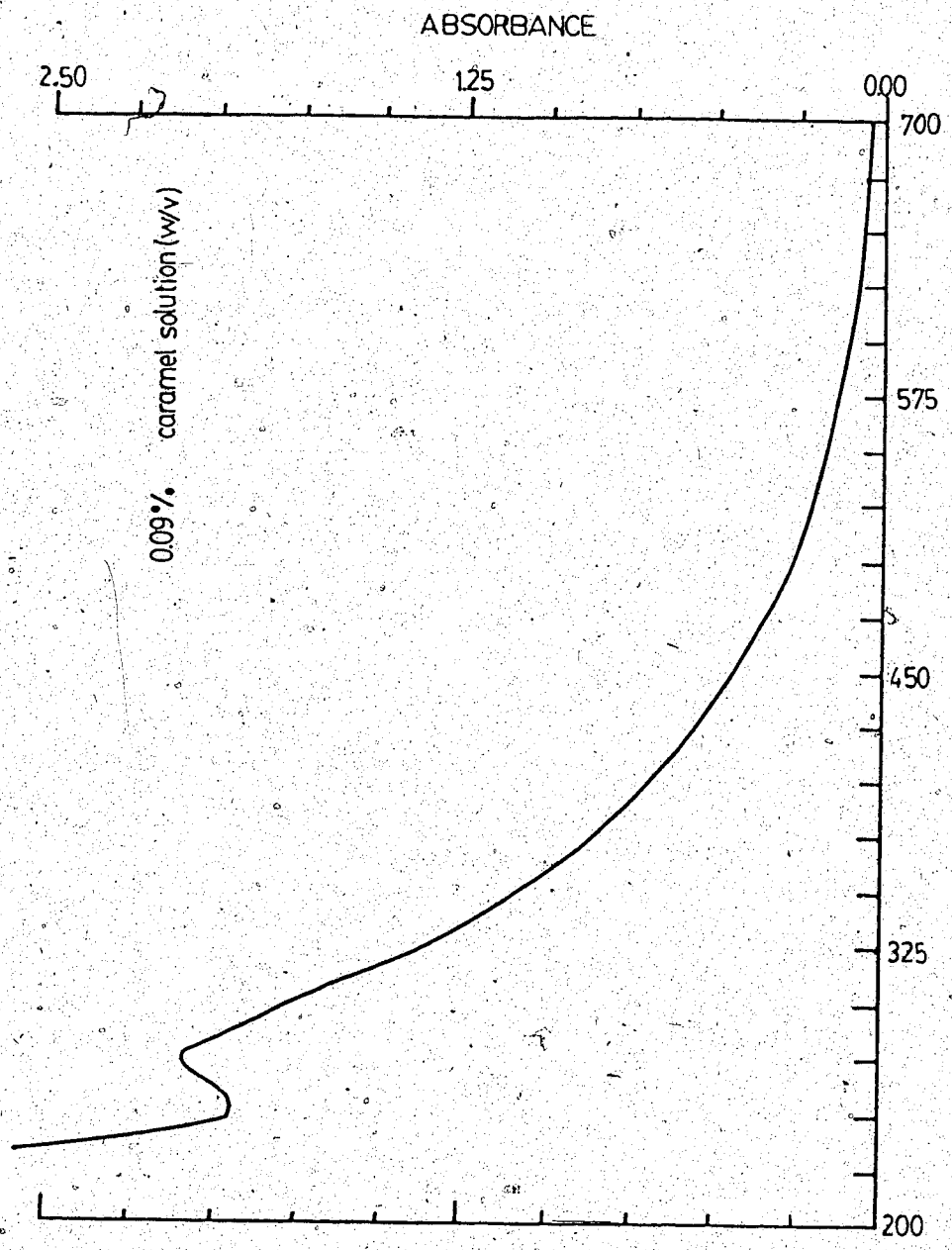


Figure 36. Wavelength scan of 0.09% (w/v) caramel solution.

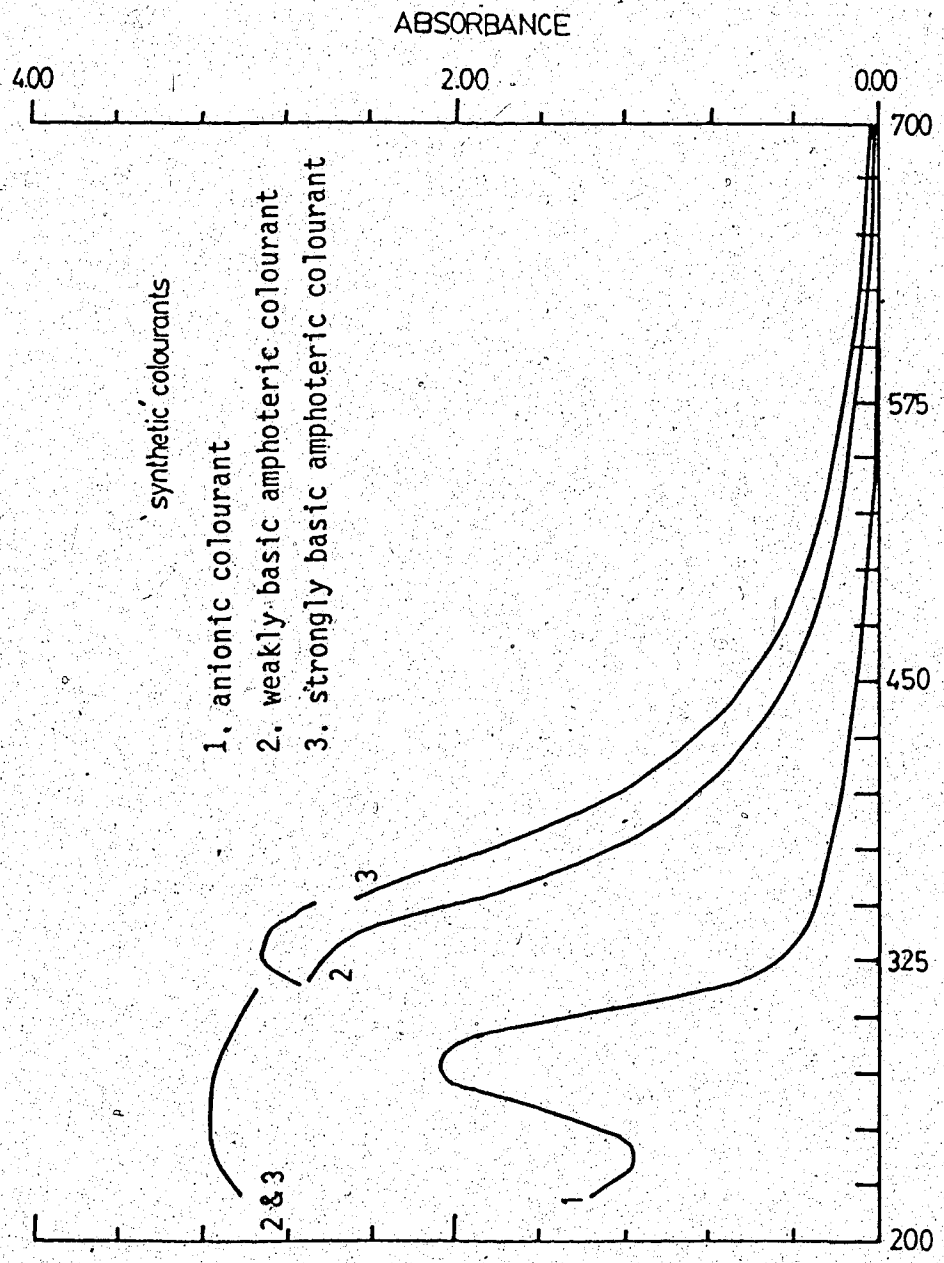


Figure 37. Wavelength scan of 'synthetic' colourants prepared according to Cookson *et al*, (1970).

peak at 275 nm (Figure 33, curve No. 2). The samples treated further with activated carbon and demineralising resins also displayed similar peak at 275 nm, but with greatly reduced absorbance values (Figure 33, curves No. 3 and 4). Colourants fractionated with Sephadex G - 25 (Figure 34) demonstrated a peak at 275 nm for both yellow and brown fractions, though that of the former was more prominent. Those fractionated with Amberlite XAD - 2 resin (Figure 35) also showed similar absorbance values, but the peaks at 275 nm were not as prominent as in the previous case. Caramel solution showed a distinctive peak at 275 nm (Figure 36). Synthetic colourants (Figure 37) demonstrated different peak maxima for different complexes. Curve No. 1, the colourant produced by heating glucose only, showed a well defined peak at 275 nm. Curves No. 2 and 3, representing colourants produced by heating glucose and lysine and glucose and glycine, respectively, showed a relatively distinctive peak at 340 nm and a rather broad peak at 275 nm.

Agarwal and Misra (1972a,b) associated the peak at 275 nm with the presence of caramel produced by heating during processing and/or drying of sugar. They reported a well defined peak at 275 nm for both cane and beet molasses. The scan of caramel solution (Figure 36) and the colourant produced by heating glucose solution (Figure 37, curve No. 1) clearly substantiated these findings. The same authors stated that melanoidins did not show peak maxima at 275 nm. However, the scan of the Maillard reaction products

(Figure 37, curves No. 2 and 3) showed absorbances at 340 and 275 nm. This could indicate that they were a mixture of melanoidins and caramel. It is interesting to note that these curves are very similar to that of the diluted molasses. Therefore, it could be concluded that colourants of Alberta beet molasses consist essentially of caramel and melanoidins, the former being predominant.

The scan of the ultrafiltrate (Figure 33, curve No. 2) showed peak maxima at 275 nm, with reduced absorbance value, and no detectable peak at 340 nm. This indicated that practically all melanoidins and some caramel were removed at this stage. After activated carbon treatment, there was a dramatic reduction of absorbance value at 275 nm (Figure 33, curve No. 3), suggesting that most of the caramel colourant was removed during this treatment. The colourant was further removed during the demineralisation step as shown by the slight reduction in the absorbance value on curve No. 4, Figure 33.

In summary, therefore, it could be stated that Alberta beet molasses colourants consisted of yellow and brown fractions. The majority of the colourants were the products of caramelisation with small contribution from Maillard reaction, occurred during sugar processing.

Another noteworthy observation in this spectrophotometric analysis was that the measurements of beet molasses colourants at 420 nm over a wide range of concentrations appeared to obey

Beer's law very well. The linear relationship between absorbance values and colour intensity of the product greatly simplified the monitoring of changes in the product at various stages of the process.

5.4 Demineralisation

Demineralisation of the ultrafiltrated and activated carbon treated molasses was the final operation in the purification of beet molasses before the production of HFS. The aim of demineralisation was to partially remove the ash in the molasses (ash present originally and those contributed by the powder carbon during the carbon treatment). Excessive ash content could reduce the capacity of the resins in the subsequent processes of hydrolysis, separation and isomerisation. Both batch and column methods of demineralisation were attempted, similar results were obtained. However, with the column method considerable problem with mono-bed resin shrinkage was encountered when the feed was introduced to the bed, causing channelling of feed. The resin column had been previously sweetened-on with the appropriate sugar concentration. This shrinkage problem could, therefore, not be due to osmotic differences between the feed and the bed. According to Khym (1974), different ions have different degrees of affinity for a particular ion exchanger. Differences in degrees of affinity for the ion exchanger could have a considerable influence upon the degree of shrinkage or swelling of the resin. The ions with the strongest affinity for a particular ion exchange

resin would produce the most shrinkage in the bed made of that resin. Since demineralisation essentially involved an ion exchange reaction whereby cations and anions of the feed solution are replaced by hydrogen and hydroxide ions, respectively, the shrinkage of the bed in the column method could most likely be due to the exchange of counter-ions on the resin. Nevertheless, a column of mono-bed resin without shrinkage was successfully made, and a breakthrough curve for the demineralisation of ultrafiltrated and activated carbon treated molasses was obtained (Figure 38). The feed had an ash content (expressed as % NaCl) of 1.15% (w/w) (See Figure 26). Four bed volumes of feed were treated before the total exchange capacity of the mono-bed resin was completely exhausted (Figure 38). In most cases, as in this one, the breakthrough curve is typified by a symmetrical S configuration. The theoretical capacity of the mono-bed exchanger was the bed volume V, corresponding to the ratio of ash content of effluent to feed (C/C_0) of 0.5. For this experiment V was found to be slightly greater than 2.

Factors affecting the capacity of an exchanger bed are the exchange potential of the ions to be exchanged, the particle size of the resin beads, the flow rate of the feed through the column, the operating temperature, the composition of the feed, and the dimensions of the column (Khym, 1974). The demineralisation by column technique was operated at ambient temperature and a flow rate of 0.88 mL/cm^2/min. Comparing both

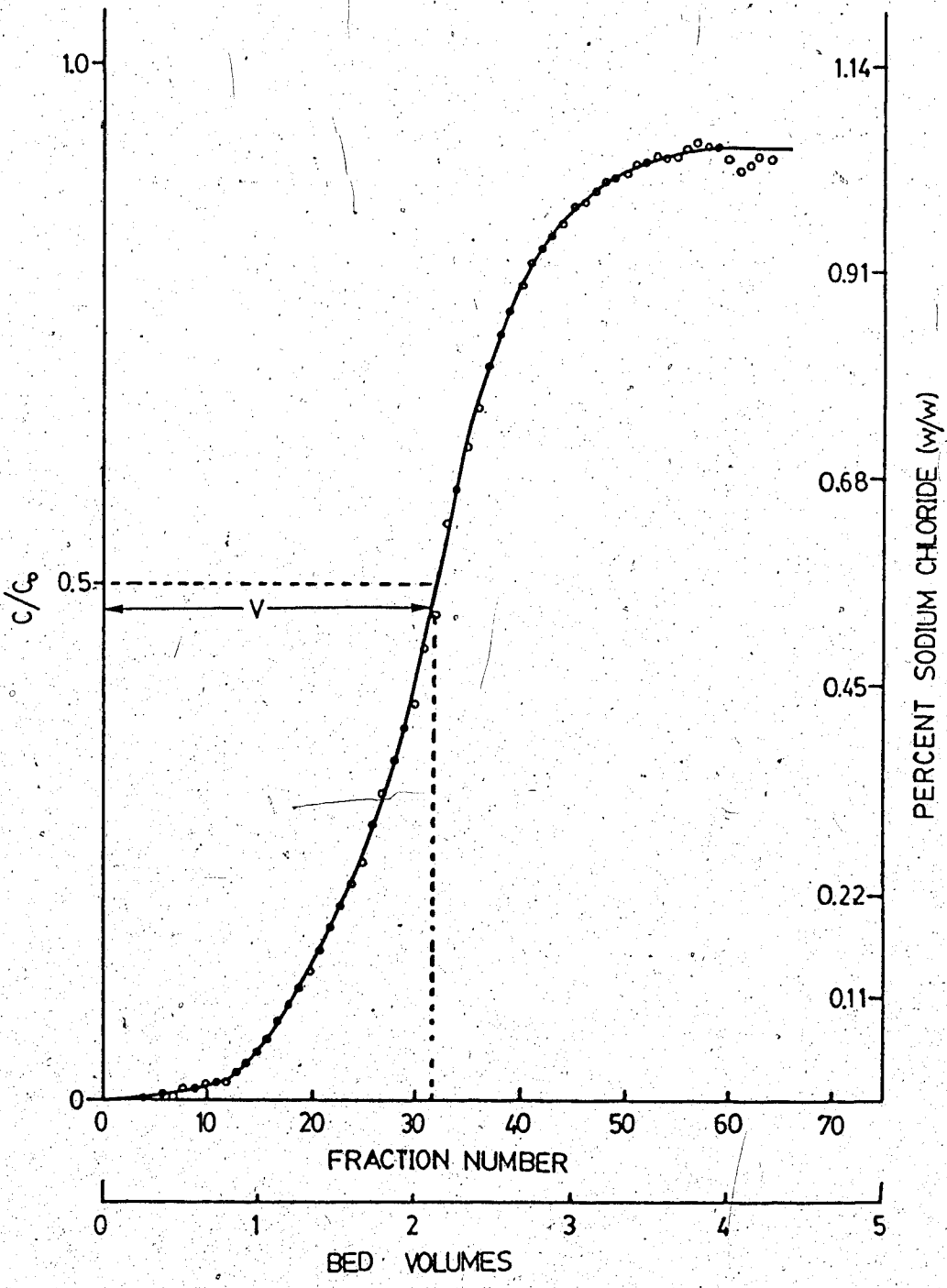


Figure 38. Break-through curve for demineralisation of ultra-filtrated and activated carbon treated molasses solution. (C_o = ash content of influent (feed), C = ash content of effluent.)

methods, the column method was more complicated but more versatile. Depending on the degree of demineralisation desired, various fractions could be collected by a system of valves and varying amounts of feed could be processed. Recycling of the effluent was also possible. The batch process was less versatile in that a 'composite' product was obtained with the degree of demineralisation dependent on the amount of resin used and the conditions employed. However, due to considerable difficulties associated with the column method, all the subsequent demineralisation operations used were batch process employing mono-bed resin added at the amount of ¼ (v/v) of the amount of the solution to be treated. A composite sample from the column method and a sample from the batch method showed a similar reduction (40,87%) of the original ash content of the feed (See Figure 26). Hence, only partial demineralisation was attained. More complete demineralisation was possible if a higher resin to feed ratio was used. However, the efficiency of the process might decrease as more resins would be required to treat a unit volume of feed. Moreover, associated problems of resin regeneration and operating costs, together with the cost of the regenerants and the disposal of used regenerants would pose a problem. On the other hand, the succeeding operations also involved synthetic organic ion exchange resins and it would be desirable to have a feed that as much of its ash removed as possible. A more highly demineralised feed for the succeeding operation of hydrolysis, separation and

isomerisation would mean longer operating cycle and reduced operating costs on an industrial scale. At this point, it is not possible to postulate what percent of demineralisation is optimum. Only a detailed process and economic analysis of the demineralisation and preceeding unit operations can give a clearer picture.

In addition to the reduction of the ash content, 4% of the original colour of the diluted molasses was also removed (See Figure 22). The removal of the colour could be attributed to both ion exchange and adsorption phenomena (Meade and Chen, 1977). The pH of the demineralised solution also decreased significantly from 7.00 to 5.00 (See Figure 25). The reason for the pH drop was not clear. A 18.62% increase in total sugar occurred during this process due to sweetening-on (See Table 3).

5.5 Hydrolysis

Hydrolysis of sucrose was the first unit operation in the production of HFS. A strong cationic resin in the H^+ form was used to sever the glycosidic bond between glucose and fructose in sucrose molecule. The strong cation exchanger in the H^+ form can be considered as a solid strong acid. This exchanger could act as a catalyst in certain aqueous reactions if the solutes present can penetrate the exchanger matrix and undergo reaction with the active counter-ion (H^+) of the exchange material. As was also stated by Hughes *et al.* (1952) ion exchange resins used in hydrolysis of sucrose should have a divinylbenzene content less

than conventional resins so as to facilitate the penetration of the resin beads by the sucrose molecules. The resin employed in the present experiment (Bio-Rad, AG 50W - X4) had a large pore size with only 4% divinylbenzene crosslinkage (See Appendix II for resin specifications). Hence, penetration of the resin by sucrose molecules should not be a problem. This was evidenced by the complete hydrolysis of the sucrose in the feed material at a feed flow rate of 0.19 mL/cm^2/min and operating temperature of 60° C. Four bed volumes of feed was passed through the column before incomplete hydrolysis occurred. The incomplete hydrolysis occurred as a result of the resin poisoning through the exchange of cations from the feed which had not been completely demineralised. This caused the release of H^+ ions into the aqueous system giving rise to the significant drop in its pH from 5.30 to 2.20 (See Figure 25). The rate of hydrolysis could be controlled by the feed rate and operating temperature. An increase in the operating temperature or a decrease in the feed rate would increase the rate of hydrolysis. However, there are drawbacks to both conditions. Prolonged exposure (i.e. slow feed flow rate) to high operating temperature could cause significant deterioration of the hydrolysate (Pigman, 1948). The hydrolysis conditions employed in this process (0.19 mL/cm^2/min at 60° C) could be a little severe as they produced a slight increase in colour (5.66% increase in O. D.) of the hydrolysate as compared to the feed (See Figure 22). However, HPLC analysis of the hydrolysate did

not detect other compounds except fructose and glucose. This was probably due to the fact that the impurities produced during the hydrolysis were removed from the sample during the treatment with a Waters C_{18} Sep-Pak cartridge. The light yellow hydrolysate was almost colourless after the filtration treatment. A major portion of the yellow colour was retained at the top of the filter bed of the cartridge as a yellow band. The yellow colour complexes could be polar and/or non-polar as the filter material in the Sep-Pak cartridges was C_{18} (Octadecylsilane) bonded to silica. Non-polar impurities would be adsorbed on the C_{18} phase and polar materials would most likely be adsorbed to the silica matrix.

A further evidence of probable degradation of the monosaccharides during the hydrolysis was a 10.46% sugar loss during the process (See Table 3). Referring to Figure 24, E, the fructose and glucose contents of feed material were 1.56 g/100 mL and 13.03 g/100 mL, respectively. As complete hydrolysis of sucrose took place, theoretically we should obtain a total of 8.08 g/100 mL of fructose and 6.52 g/100 mL of glucose. However, the actual sugar contents found in the hydrolysate were 7.15% for fructose and 5.91% for glucose (Figure 39, F) indicating a loss of 11.51% and 9.36%, respectively. The loss of fructose was greater than of glucose, probably due to it being more reactive.

Another interesting observation was that colour compounds originally present in the feed was retained in the

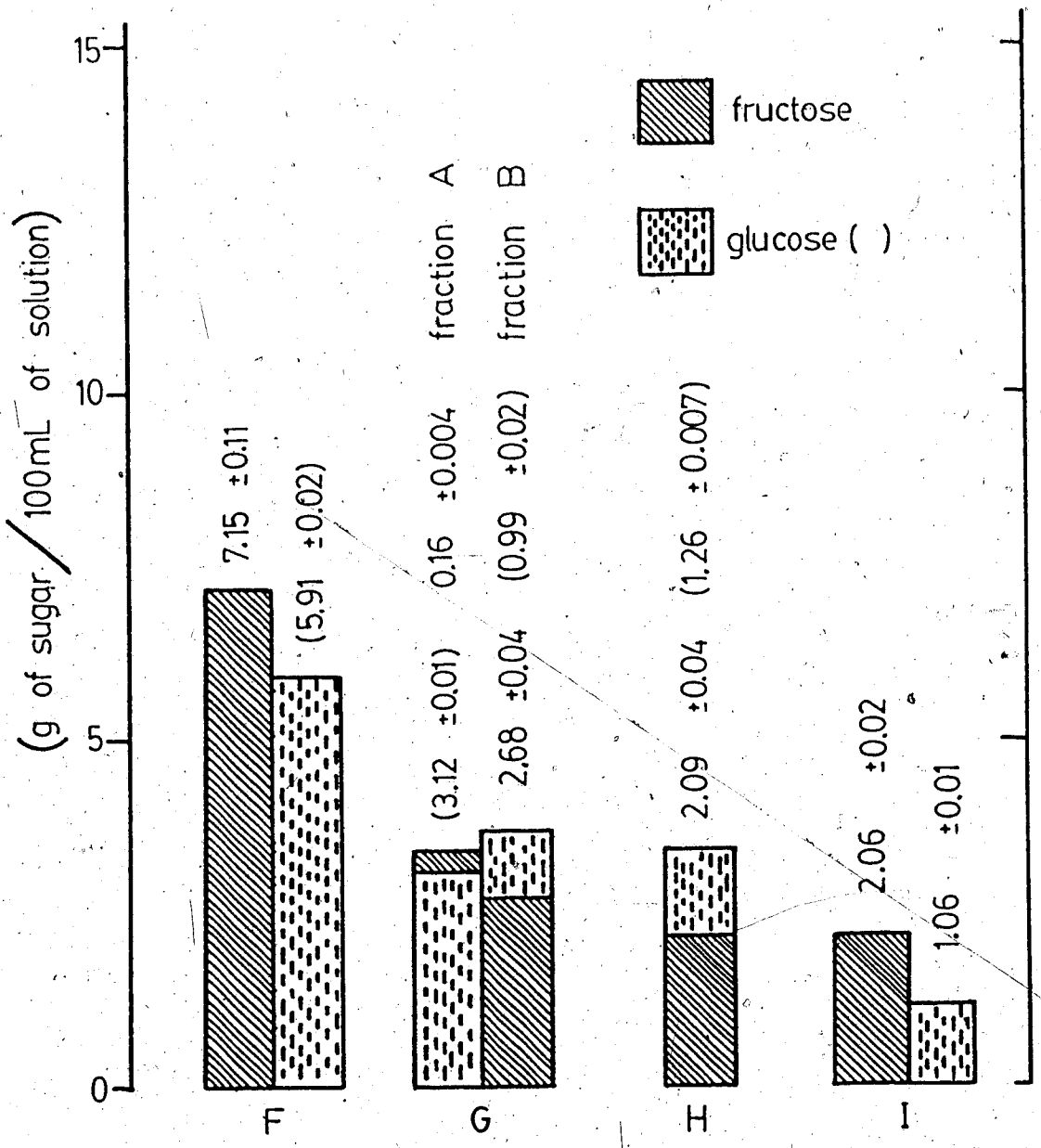


Figure 39. Sugar profile of the product after each unit operation in high fructose syrup production. F - hydrolysate, G - fractions A (glucose) & B (fructose), H - isomerised product, I - final product (fraction B + isomerised product)

top layers of the resin bed, while those formed during hydrolysis was never retained on the column. This points to the difference in the chemical and/or physical natures between the hydrolysis-induced colour compounds and those originally exsisting in the molasses.

5.6 Separation

The separation process was carried out to separate glucose from fructose so that glucose could be subsequently isomerised to increase the fructose content of the final product (HFS). A calcium-charged resin was used in the separation of the monosaccharides in the hydrolysate. Partial separation of glucose from fructose was attained (Figure 40) with glucose eluting before fructose. Petersen (1975) stated that resolution of monosaccharides depended on operating variables in the separation process such as eluant flow rate, size of the resin employed, operating temperature and sample load size. When the following conditions were used: 0.25 mL/cm^2/min eluant flow rate, feed load for each cycle of 10% of bed volume, and operating temperature of 60° C, the resolution obtained was 0.57 signifying only partial separation of the monosaccharides. A decrease in eluant flow rate, size of resin and feed load, or an increase in operating temperature should increase the degree of resolution. However, suitable conditions to be used in the industry would depend on the operating characteristics of each plant and the desired

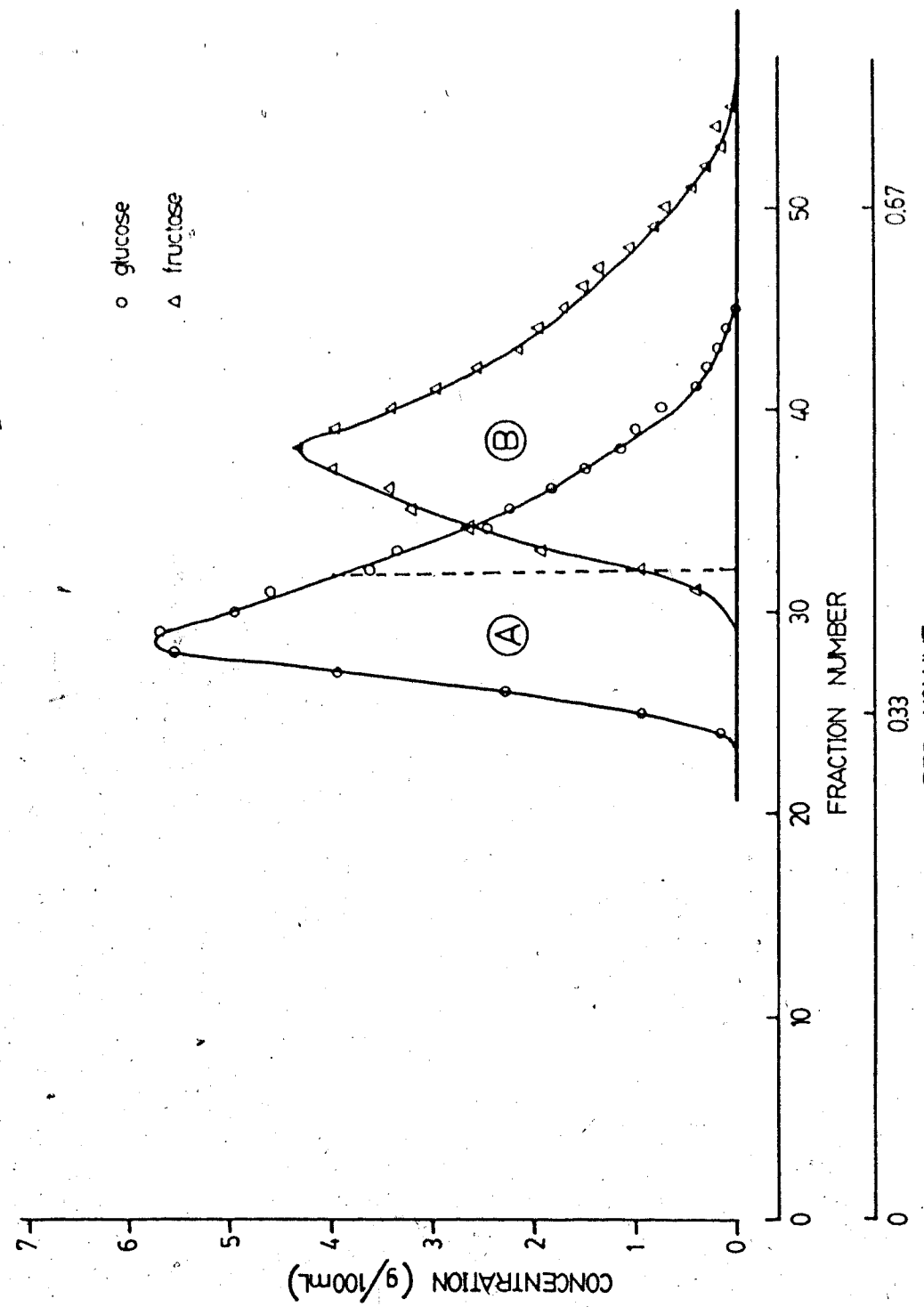


Figure 40. Separation of glucose from fructose with a calcium-charged resin.
Ⓐ - glucose fraction; Ⓑ - fructose fraction

end product.

As can be seen from Figure 40, various combinations of pure or mixtures of fructose and glucose syrup could be obtained by employing a system of valves to collect and combine various fractions to obtain the desired product composition. In industry, recycling of the various fractions through a complex system of valves and pumps is possible to attain a better degree of resolution (Petersen, 1975; Johnson, 1976). The sample loading factor is determined by the capacity of the column, i.e. the exchange capacity and volume of the resin used. The peak volume for glucose, which preceeded fructose in the elution, was between the 28th and 29th fractions and that for fructose was at the 38th fraction.

According to Khym (1974) a void volume for spherical beads, packed in a column with a diameter of $\geq$ 10 times that of the beads, is approximately 40% of the total bed volume. The glucose elution volume obtained in this experiment was approximately 38 mL, which is very close to the void volume. This implies that there was almost no retention of glucose by the resin, i.e. little or no complex formation between glucose and bed materials occurred. Fructose, on the other hand, took longer to elute through the column. This could be due, to some degree, to the complex formation between fructose and Ca^{2+} on the resin, e.g. the formation of an adduct of calcium fructosate (Petersen, 1975; Johnson, 1976). Briggs *et al.* (1981) investigated the use of

thin layer chromatography as a simple means of resolution of mixtures of carbohydrates after complex formation with metal ions such as Cu^{2+} and Ca^{2+}. They reported that D-fructose formed polyhydroxy compound with Ca^{2+} more easily than D-glucose, and the complex was also more stable. In aqueous solution the complexing reaction is reversible. Therefore, the complexed fructose could be uncomplexed or vice versa depending on the surrounding conditions, until an equilibrium is reached. The same authors also reported that about 9.1% of fructose in the solution would form complexes as compared to only 1% of glucose. This seems to explain the resolution behaviour of the monosaccharides in the separation process quite well.

Fraction A of separation product consisted of fractions 22 to 32 inclusive, and had the following characteristics: an absorbance value of 0.221 (See Figure 22), a pH of 4.52 (See Figure 25), and glucose and fructose contents of 3.12 g/100 mL and 0.16 g/100 mL, respectively (See Figure 39, G). The ratio by weight of glucose to fructose was 20 to 1.

Fraction B consisted of fractions 33 to 55 inclusive, and had the following characteristics: an absorbance value of 0.064 (See Figure 22), a pH of 5.25 (See Figure 25), and glucose and fructose contents of 0.99 g/100 mL and 2.68 g/100 mL, respectively (See Figure 39, G). The ratio by weight of glucose to fructose was 1.00 to 2.71.

During the separation process there was about 4½ fold

dilution of the product. The feed had a glucose and fructose concentration of 5.91 g/100 mL and 7.15 g/100 mL, respectively (See Figure 39, F). From Figure 40, the maximum concentration of glucose obtainable with the separation conditions employed was 5.75 g/100 mL, hence, a dilution of 2.71%. The maximum concentration of fructose obtainable was 4.30 g/100 mL, a dilution of 39.86%. Therefore, fructose was diluted approximately 14½ times more than glucose. This was most probably due to the greater residence time of fructose in the column as compared to glucose, thus getting eluted with greater volume of eluant.

The choice of the separation conditons adopted would vary according to desired results. A higher resolution of a mixture could possibly be attained at the expense of processing time. The most potent determining factor would most likely be the type or types of the product warranted by the market situation. The sugar gain in this process was 22.71% (See Table 3).

5.7 Isomerisation

Isomerisation was carried out to increase the fructose content of the final product. Isomerisation of the glucose feed (Fraction A) was performed in a chromatography column using a strong base anionic exchange resin in OH^- form. The feed flow rate was 0.10 mL/cm^2/min and operating temperature was 55° C. The glucose content of the feed was 3.12 g/100 mL and fructose content was 0.16 g/100 mL. The isomerised product had the

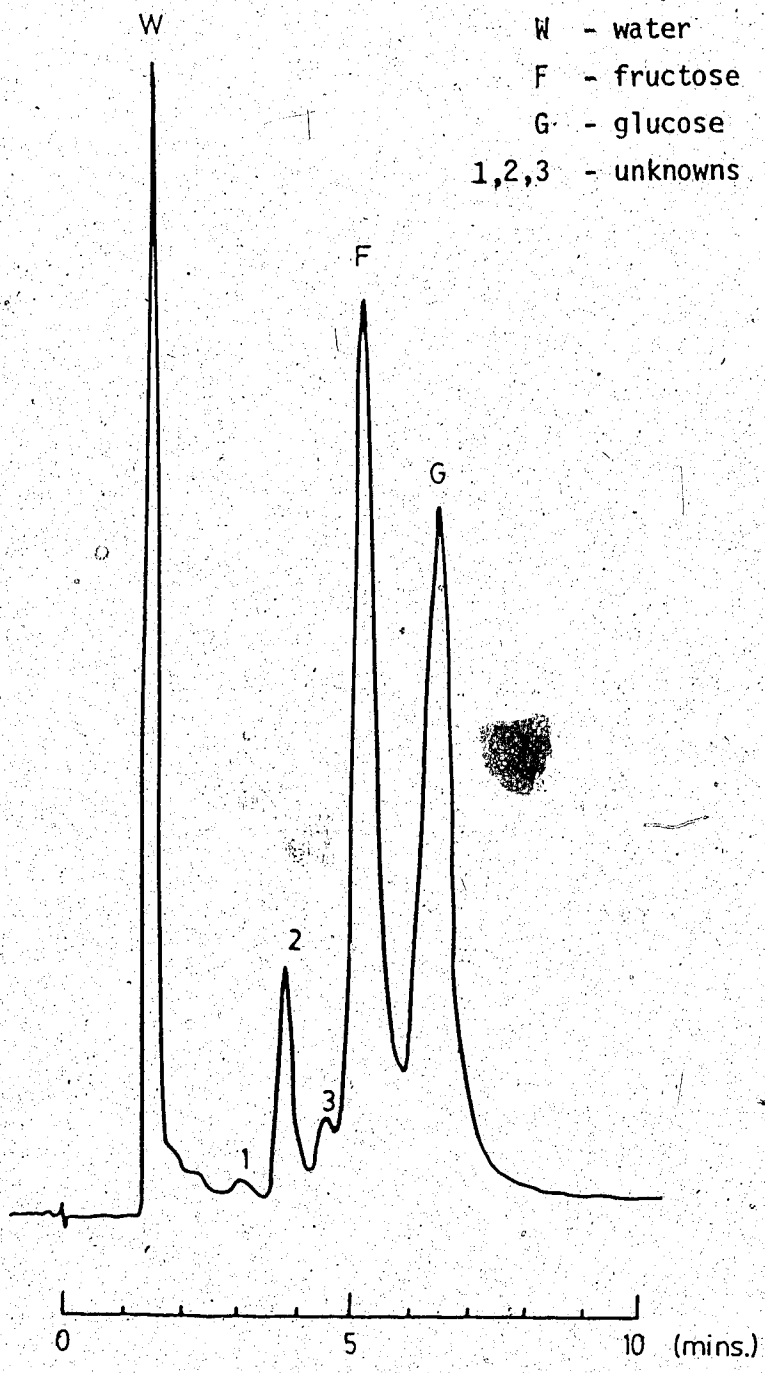


Figure 41. A typical HPLC chromatogram of isomerised product.

following characteristics: an absorbance value of 0.200, a pH of 8.75 (See Figure 25), glucose and fructose contents of 1.26 g/100 mL and 2.09 g/100 mL, respectively (See Figure 39, H). This represented the isomerisation ratio (fructose content in product, X 100 / total sugar in product) of 62.39%.

When the resin bed was sweetened-on its colour changed from light brownish yellow to dark brown. However, this did not appear to affect the colour of the product which remained largely unchanged throughout the process. Regeneration of the resin with NaOH released much of the dark brown colour into the solution, but never restored the resin to its original colour. The colourants could be the result of the degradation of monosaccharides at high pH and temperature (Pigman, 1948). The 2.40% gain of sugar during the isomerisation process (See Table 3) could be attributed to the sweetening-on process.

The degradation of sugars also gave rise to the presence of small quantities of by-products in the isomerised solution (Figure 41, peaks 1, 2 and 3). The identities of the by-products have not been elucidated. However, according to Conrad and Palmer (1976) one of them, either peak 1 or 2, could most likely be D-psicose (D-allulose). Another possible alkaline isomerisation by-product, D-mannose, was not detected. Honig (1953) stated that when strong bases such as NaOH were used in sugar isomerisation fructose would be produced, whereas when weak bases such as $Ca(OH)_2$ were used D-mannose would likely be the

product instead of fructose.

Isomerisation reaction in an alkaline condition is dependent on pH and temperature. Higher pH and temperature will produce higher isomerisation ratio, but with greater amounts of by-products (Johnson, 1976). The isomerisation reaction is an equilibrium reaction in which the product, fructose, has an effect on the final concentration. Many attempts had been made to increase the fructose content in the product. One of these is the addition of borates to complex the fructose being formed so as to shift the equilibrium towards the production of more fructose (Johnson, 1976). In this experiment a typical isomerisation ratio obtained was 62.39% which is comparable to that obtained by Johnson (1976).

6. SUMMARY

Considering this study as a whole, therefore, it could be concluded that conversion of Alberta beet molasses to HFS is technically feasible, using the process as proposed. However, the overall economics of the process will decide whether it will be used industrially. The processes of activated carbon treatment and demineralisation, hydrolysis, separation and isomerisation with synthetic resins are well established and numerous patents on the subject have been issued. The ultrafiltration technique as applied to diluted beet molasses is new and much more research to understand the underlying principles, the performance, the deficiencies and the economics of the process should be done. The 'bottle-neck' experienced in this process could be related to ultrafiltration. However, since only the plate and frame model design was used, it could not be concluded that the process is not suitable for molasses. Other designs such as tubular, spiral wound, etc., must be tried before any general conclusions are drawn. Another possibility is to use ultrafiltration as a supplement to other decolourisation processes rather than as the first unit operation in the process. This may improve its efficiency.

Synthetic organic resins used in demineralisation, hydrolysis, separation and isomerisation have the advantage of regenerability. When compared to enzymes they have a much longer

life span. It is the nature of the substrate which will dictate whether enzymes or resins are to be used in the production of HFS from beet molasses. The substrate, even after 3 purification steps, is not sufficiently pure or clean for further processing by the enzymes presently available. Of course, if a 'super-tolerant' enzyme could be produced the situation might change.

The final product, high fructose sugar solution, was a combination of fraction B from the separation step and the product from the isomerisation process. It had the following characteristics: a clear light yellow colour of 0.125 Absorbance unit (See Figure 22); a pH of 5.72 (See Figure 25); a total sugar content of 3.12% (2.06% fructose and 1.06% glucose) (See Figure 39, I); and, a total solids content of 3.25%. The product tasted sweet with a slight sour after-taste and had a slight residual odour of beet molasses. The fructose content of the final product was 66.0% by weight of total sugar and, hence, could be suitably concentrated to a high fructose syrup (HFS).

Depending on what the product is intended for, further treatments, apart from concentration, may include decolourisation, addition of preservatives and deodourisation. Conventional concentration processes such as multi-stage evaporation, reverse osmosis or freeze concentration may be used. However, colour development may be a problem if the process involves high temperature. Vacuum evaporation at relatively low temperature may minimise the problem of colour development and provide

added benefit of odour removal. Reverse osmosis has been used to concentrate products such as whey protein but may not be viable for this product because of the high capital and operating cost involved. Further decolourisation with activated carbon and/or synthetic resins is a possibility. The addition of preservatives is unnecessary if the product is concentrated to greater than 40% t.s. With its high fructose content, crystallisation of the syrup will not be a problem, and hence it may be advantageous to concentrate the product as much as possible to improve storage stability and reduce handling, storage and transportation costs.

As a sweetener the residual molasses odour and the slight sour after-taste require special attention in devising further treatments. The colour of the product is acceptable if it is used as a table-top syrup or as an ingredient in the manufacture of other products such as canned or baked goods. For the beverage industry, however, proper decolourisation and deodourisation of the product are necessary.

The economic feasibility of the proposed process depends essentially on the price of sweeteners from other processes such as the traditional cane and beet sugar industry, the high fructose corn syrup industry and other competitive sweeteners such as artificial sweeteners. Intensive research is under way in the field of bioengineering through enzymology to develop ways to utilise the abundant supply of cellulose material to produce glucose syrup which can be isomerised to HFS (Gramera, 1978).

This can be a very attractive source of sweetener in the future.

It may be argued that the use of beet molasses as a raw material for the production of HFS is a poor choice since it can be converted to many other valuable commodities, e.g. rum, important chemicals and pharmaceuticals, etc. However, this study was initiated to show if Alberta beet molasses, which has had rather limited use heretofore, could be converted to HFS. Towards that goal, the study has demonstrated that it is technically possible. Whether it is economically viable depends on the industries concerned and the future market situation.

SUGGESTIONS FOR FUTURE RESEARCH.

There are several areas of academic as well as practical interest which require more research. These include:

1. Elucidation of chemical and physical natures of colouring components and other impurities in Alberta beet molasses. This would aid in the design of more efficient purification processes.

2. More thorough studies of ultrafiltration as a unit operation in the removal of colour components and impurities in the molasses.

3. Ultrafiltration membranes most suitable for the removal of molasses colourants and impurities. Their kinetic behaviours during filtration, fouling effect and means to minimise or rectify it.

4. The use of enzymes as an alternative method to hydrolyse and isomerise purified molasses.

5. Pilot plant studies of the proposed process.

6. Further refinement and concentration of the product to suit various uses.

7. Economic evaluation of the process.

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APPENDIX I

Washing and regeneration procedure for exhausted resin. (Batch method).

a. The 'exhausted' resin was first transferred to a 1000 mL beaker and excess sugar or feed solution removed by decantation.

b. Sufficient quantity of distilled water was warmed to about 50° C in a separate beaker.

c. The resin was then mixed with the warmed water (approx 1:2) and stirred on a hotplate/stirrer for 5 to 10 minutes before decanting. Resin fines which might have accumulated during the experiment were also removed at this stage.

d. Washing and decanting were repeated several times until the supernatant was free or had only trace of soluble solids as checked by a pocket refractometer.

e. The resin was then slurried in distilled water and filtered through a Buchner funnel.

f. The semi-moist resin was transferred from the funnel into a 1000 mL beaker and washed with either ethanol or methanol by stirring the resin-alcohol mixture slowly on a magnetic stirrer for 5 to 10 minutes before decanting the supernatant.

g. The alcohol washing process was repeated two or three times before the final washing with distilled water and regeneration.

h. For an ANIONIC resin, a single washing with a 5% w/v solution of an acid (eg. HCl) was desirable to remove any remaining basic compounds before the regeneration step.

i. For a CATIONIC resin, a washing with 5% w/v solution of a base (eg. NaOH) was desirable to remove any remaining acidic compounds before the regeneration step.

j. A 5% w/v solution of NaOH was used for the regeneration of ANIONIC resins if the OH^- form was desired, while a 5% w/v solution of HCl was used for CATIONIC resins if the H^+ form was desired.

k. Repeated stirring of the resin with the regenerant on a magnetic stirrer followed by decantation was performed until the resin was completely regenerated. Substantial excess of regenerant was usually used to ensure that the resins were well regenerated as there was no straight forward and fast method, besides titration, to check if the regeneration was complete.

l. The regenerated resins were then washed with copious quantity of distilled water. To ensure that the resins were completely free of the regenerant, the pH of the supernatant of the

washing process was checked. The washing was terminated when the pH of the supernatant was less than 8 for ANIONIC resin and more than 6 for CATIONIC resin.

m. The regenerated resins were finally slurried in distilled water to assist in the transfer to a Buchner funnel where they were filtered under a vacuum and stored in the semi-moist condition.

Washing and regeneration procedure for exhausted resin. (Column method).

Column washing method was more effective than the batch method as less distilled water and regenerants were required to attain the same degree of washing. However, it took considerably more time to accomplish.

a. Distilled water was warmed in a beaker to a temperature of 50°C and was transferred to the R 15 or R 25 reservoir of the chromatography column.

b. The 3-way valve was switched to direct the flow of the eluent through the resin bed in the column.*

c. The flow rate can be adjusted to any desired value by raising or lowering the reservoirs. Usually 2 to 5 bed volumes per hour was adequate for this 'sweetening-off' and washing process.

d. Operating the column at a temperature higher than ambient also assisted in the washing operation.

e. The effluent was checked periodically for soluble solids (mostly sugars) with a refractometer. When the sugar was completely removed, the flow was terminated.

f. In the regeneration step either ethanol or methanol was used as eluent.

g. The alcohol washing was performed at ambient temperature at the flow rate of about 2 to 3 bed volumes per hour. The washing assisted in removing any organic impurities that may have been sorbed by the resins.

h. Most alcohol washing requires 3 to 4 bed volumes of alcohol, followed by distilled water to remove traces of the alcohol before proceeding to the next step in the regeneration process.

i. As was with the batch process, an ANIONIC resin was given an acid wash and the CATIONIC resin a base washing before the regeneration proper.

j. For an ANIONIC resin a 5% w/v solution of NaOH was used with a flow rate of about 5 bed volumes per hour totalling between 10 to 15 bed volumes. Similar flow rates and regenerant quantities of 5% w/v HCl were used for CATIONIC resin.

k. The regenerated resins were washed with distilled water at flow rates similar to that of the regenerant. The washing was terminated when the pH of the effluent was <8 for ANIONIC and >6 for CATIONIC resin.

* It was important to loosen an end flow adapter of the column at the initial stage of the washing operation as the resin could swell considerably and rupture the column especially when the preceeding feed solution was quite concentrated.

Regeneration of 'mono-bed' resin

The regeneration procedure for a 'mono-bed' resin of H^+ and OH^- forms was similar to that of ANIONIC and CATIONIC resins as outlined in the preceeding section. However, the CATIONIC resin (H^+ form) must be first separated from the ANIONIC resin (OH^- form). This was done by suspending the mixture in a 30% w/v solution of NaCl. The ANIONIC resin being lighter would float to the top and could be separated from the heavier CATIONIC resin by careful decantation. The resins were then regenerated, rinsed and remixed in a beaker of distilled water.

Resin washing procedure of 'new' CATIONIC resin in the H^+ form used in the hydrolysis process.

The resin washing procedure for 'new' H^+ resin was similar to that of exhausted resin for both batch and column methods, and the washing was terminated when the pH of the wash water >6.

Conversion of the Cl^- form to the OH^- form.

An ANIONIC resin in the OH^- form was required in the isomerisation step. However, the resin supplied by Bio-Rad Laboratories (Canada) was in the Cl^- form. Hence, a conversion of the Cl^- to the OH^- form was required. The steps in the conversion were as followed:

a. The resin was slurried in distilled water and packed into a Pharmacia K 26/40 chromatography column as outlined in the 'resin packing procedure' section.

b. A 5% w/v NaOH solution was passed through the resin bed at ambient temperature. The flow rate varied between 2 to 3 bed volumes per hour.

c. The effluent was checked for the Cl^- ion with 0.1N $AgNO_3$ solution. A white precipitate indicated the presence of the Cl^- ion signifying an incomplete conversion of the resin to the OH^- form. A yellow or light brown precipitate indicated that the bromide ion was present or that the solution was too basic.

d. The elution was terminated when the effluent was free of Cl^-. Normally, 12 to 15 bed volumes of feed was required for complete conversion of the Cl^- to the OH^- form.

Conversion of the H^+ form to the Ca^{2+} form.

A CATIONIC resin in the Ca^{2+} form was required in the separation step. This was obtained by converting the H^+ resin to the Ca^{2+} form. The steps in the conversion were as followed:

a. The H^+ resin was slurried in distilled water and packed into a Pharmacia K 26/40 chromatography column.

b. The column temperature was maintained at 60° C by means of a water bath and a circulator.

c. A 10% w/v $CaCl_2$ solution was passed through the resin bed at an approximate flow rate of 3 bed volumes per hour.

d. The pH of the effluent was monitored. The feed was terminated when the pH of the effluent was similar to that of the feed. Complete conversion required between 10 to 15 bed volumes of feed.

e. The resin was then washed with distilled water until the effluent was free of the Cl^- ion.
(See preceeding section for test on Cl^- ion).

Resin packing procedure with the 'slurry' technique.

a. 100 mL of resin in distilled water was measured into a measuring cylinder. The resin was allowed to rehydrate fully and settled properly.

b. The bottom end flow adapter of the chromatography column was inserted into the inner glass column before the resin was transferred into the column.

c. A small amount of distilled water (approximately 5 to 10 mL depending on the diameter of the column) was poured on top of the flow adapter.

d. The fully hydrated and measured resin was carefully poured into the column in 5 to 6 sections by gently swirling the resin during the pouring process to ensure that the resin was in suspension and flowed as a slurry. Hence, the name 'slurry' technique.

e. Resin particles on the sides of the column were washed down with a wash bottle.

f. The freshly poured section was allowed to settle before the next section was poured. The supernatant was removed when necessary and additional slurry added until the desired amount of resin had been poured, leaving about 2.5 cm. of liquid above its surface.

g. Air bubbles were removed by tapping on the walls of the column. If this failed, backwashing the resin by reversing the flow through the column might help.

h. An overall visual examination of the entire column packing was performed before the top end flow adapter was inserted.

i. Mixed bed (mono-bed) columns were poured in very short sections as the cation resin, being denser than the anion resin, would settle faster resulting in an irregular resin distribution.

j. For resins that require 'sweetening-on' the steps from a to i preceeded sweetening-on process. Due to osmotic effects, the resins would shrink and the column end flow adapters might need readjustment to obtain a properly packed bed.

'Sweetening-on' procedure for resins (Batch method).

a. The new or regenerated resin was transferred to a 1000 mL beaker.

b. Sugar solution of appropriate concentration was added to the resin. The mixture was stirred slowly for 15 to 20 minutes on a hot plate/stirrer.

c. The resin mixture was then decanted and the supernatant checked with a pocket refractometer.

d. The procedure (b to c) was repeated several times until the soluble solids concentration of the supernatant was similar to the sugar solution, signifying that the resin was ready for use.

'Sweetening-on' procedure for resins.
(Column method)

a. A measured volume of new or regenerated resin was transferred to a chromatography column according to the 'resin packing procedure' section.

b. The end flow adapters of the column were adjusted to form a tightly packed bed.

c. Sugar solution of appropriate concentration was fed through the column.

d. The effluent was checked with a pocket refractometer and the flow of the sugar solution was terminated when the soluble solids concentration of the effluent was similar to that of the feed. A flow rate of 2 to 4 bed volumes per hour was usually used. If facilities permit the operation should be performed at temperatures greater than ambient, this would assist in speeding up the 'sweetening-on' operation.

e. After the 'sweetening-on' procedure had been completed, the end flow adapters of the chromatography column might have to be readjusted to form a well packed bed as the resin bed could shrink due to osmotic effects during the 'sweetening-on' process.

APPENDIX II

Resin used in the demineralisation process:

- mixed bed or 'mono-bed'.
- Cat. No. # R-208
- REXYN I-300
- Active groups = RSO_3^- & R_4N^+
- Ionic forms = H^+/OH^-
- Mesh size = 16-50
- Moisture content = 54.5%
- Total Exchange capacity
  Wet volume = 0.78 meq/mL
  Dry weight = 1.88 meq/ g
- Fisher Scientific Company, Fair Lawn, N.J.

Resin used in the hydrolysis process;

- Analytical grade cation exchange resin
- AG 50W - X4
- Cat. No. # 142-1341
- Styrene type - sulfonic acid, effective pore size - large
- Ionic form = H^+
- Mesh size = 100 - 200
- Moisture content = 64 - 72%
- Total exchange capacity
  Wet volume = 1.20 meq/mL
  Dry weight = 5.20 meq/g
- Bio-Rad Laboratories (Canada) Ltd.,
  Mississauga,
  Ont. L4V 1H3

Resin used in the separation process:

- Analytical grade cation exchange resin
- AG 50W - X4
- Cat. No. # 142 - 1341
- Styrene type - sulfonic acid, effective pore size - large
- Ionic form = Ca^{2+}
  (H^{+} form converted to Ca^{2+} form.)
- Mesh size = 100 - 200
- Moisture content = 64 - 72%
- Total exchange capacity
  Wet volume = 1.20 meq/mL
  Dry Weight = 5.20 meq/g
- Bio-Rad Laboratories (Canada) Ltd.,
  Mississauga,
  Ont. L4V 1H3.

Resin used in the isomerisation process:

- Analytical grade anion exchange resin
- AG 1 - X4
- Cat. No. # 140 - 1341
- Styrene type - quaternary ammonium, effective pore size - large
- Ionic form = OH^-
  (Cl^- form converted to OH^-.)
- Mesh size = 100 - 200
- Moisture content = 59 - 65%
- Total exchange capacity
  Wet volume = 1.20 meq/mL
  Dry weight = 3.50 meq/g
- Bio-Rad Laboratories (Canada) Ltd.,
  Mississauga,
  Ont. L4V 1H3.

Resins used in the fractionation of molasses colourants:

A. - Amberlite XAD - 2

- Cat. No. # 40820

- An adsorbent, unspecific adsorbent based on polystyrene, high porosity is attained by a structure formed from an agglomerate of a large number of small polystyrene beads, supplied in bead form.

- Practical grade

- Mesh size = 16 - 50

- Pore size = 9 nm

- Specific area = 330 m^2/g

- Terochem Laboratories Ltd.,
  Edmonton,
  Alta.

B. - Sephadex G - 25

- Cat. No. # 17 - 0034 - 01

- Coarse size, particle size 100 - 300 μ

- Polydextran beads, fractionation range = 100 - 5000 Daltons

- Water regain (g/g dry gel) = 2.5 ± 0.2

- Approximate swelling time = 6 hr at room temperature

- Pharmacia Canada Ltd.,
  Dorval,
  P. Q. H9P 1H6

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